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3B-Hydroxysteroid Dehydrogenase Activity in the Adrenal Gland of the Chick Embryo

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3β-HYDROXYSTEROID DEHYDROGENASE ACTIVITY IN THE ADRENAL GLAND OF THE CHICK EMBRYO

by

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BIOGRAPHY

Grover Charles Ericson was born February 17, 1941, in Oak Park, Illinois.

He was graduated from Naperville Community High School, Naperville, Illinois in June, 1959. He continued his studies at North Central College in Naperville where he was awarded the Bachelor of Arts degree in June, 1964.

In September, 1964, he began his graduate studies in the Department of Anatomy, Loyola University of Chicago, Stritch School of Medicine, Maywood, Illinois. He was awarded the degree of Master of Science in February, 1968. He was a Graduate Teaching Assistant in the Department from 1965 to 1972. From 1968 to 1972 he served as a Research Assistant to Professor L.V. Domm. In the summer of 1970 and 1972, he was awarded a National Science Foundation Summer Traineeship.

While attending North Central College, Grover Ericson married a fellow student, the former Barbara Joye Moede. They have a daughter, Brenna Joye, born September 12, 1964, and a son, Gregory Charles, born June 12, 1969.

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ABSTRACT

The adrenal glands of white and brown Leghorn embryos and chicks were examined for histochemically demonstrable 3β-hydroxysteroid:NAD oxidoreductase (3β-hydroxysteroid dehydrogenase or 3β-HSD) using pregnenolone (P) and dehydroepiandrosterone (DHA) as substrates. Traces of P- and DHA-3β-HSD activity were seen as early as stage 22 (3.5 - 4 days) in adrenocortical cells of normal embryos. These activities gradually increased through eight days of incubation and were evenly distributed throughout the adrenocortical tissue; however, P-3β-HSD was always greater than DHA-3β-HSD activity. Beginning at stage 30 (6.5 - 7 days) the first indications of a zonation were observed in the adrenals of some embryos. From nine days through hatching and during the first two weeks after hatching, the adrenal glands showed a narrow peripheral cortical zone of high P-3β-HSD and low DHA-3β-HSD and a large central zone of low P-3β-HSD and high DHA-3β-HSD activity. These observations suggest the presence of two substrate-specific 3β-hydroxysteroid dehydrogenases. No differences were observed in the distribution or degree of activity of DHA- and P-3β-HSD between white and brown Leghorn embryos or chicks.

Hypophysectomy by the partial decapitation method of Fugo ('40) at stages 10 or 11 (33 to 45 hours), had no effect on the morphological development of the adrenal glands from stage 22 through nine days of incubation. Beginning at about ten days and continuing through the remainder of the incubation period, the cortical cords of hypophysectomized embryos were hypertrophied and reduced in number and the size of the groups of chromaffin (medullary) cells were larger than normal. Hypophysectomy had no effect on DHA- or P-3β-HSD activity from stage 22 through eight days of incubation. However,

after this period many of the adrenal glands of hypophysectomized embryos lacked cortical zones and when these did occur the peripheral was wider and the central zone smaller than those of normal or sham operated embryos. From eight through 19 days, the central zone P-3β-HSD activity of hypophysectomized embryos was higher than that of sham operated embryos and from 12 through 19 days the peripheral zone activity was lower than that of controls. Central zone DHA-3β-HSD activity of hypophysectomized embryos was equal to that of sham operated controls from eight through 12 days but after this period the central zone activity was lower than in controls. The peripheral zone DHA-3β-HSD activity of hypophysectomized embryos was equal to that of controls from eight through 19 days.

When hypophysectomized 8.5 day old embryos received single, whole adenohypophyses from 17 day donor embryos, transplanted on the chorioallantoic membrane, all of the adrenal glands showed zonations from nine through 19 days and the size of the zones appeared to be about normal. The P- and DHA-3β-HSD activity levels in both the peripheral and central zone were at levels characteristic of embryos with intact pituitary glands. The distribution and levels of 3β-HSD activity in the adrenal glands of hypophysectomized embryos, which had received daily administration of ACTH on the chorioallantoic membrane beginning at eight days, were also characteristic of intact embryos.

The results of this investigation indicate that the adrenocortical cells of the chick have a steroidogenic potential as soon as they become recognizable early in development. Cortical zones begin to appear on the eighth day of incubation and are seen in all adrenal glands by nine days. Following hypophysectomy there appeared to be either a retardation or a failure in the development of the central cortical zone. Adenohypophyseal transplants, as well as the administration of ACTH, prevent the effects of hypophysectomy.

INTRODUCTION

The adrenal glands of the domestic fowl are bilateral structures situated medial to the anterior lobe of the kidneys in the area of the gonads. The glands consist of two types of cords of cells, the cortical and medullary cords, which are irregularly intermingled. The cortical cords make up the bulk of the gland while the medullary cords occur in the spaces between the cortical cords. The cortical (interrenal) cells arise from the peritoneal epithelium and are seen as early as the fourth day of incubation. The medullary (chromaffin) cells arise on the sixth day from cells migrating from the sympathetic trunk which in turn originated from the neural crest.

A number of investigations have shown that the avian adrenal is dependent on the anterior lobe of the pituitary during the embryonic period. Fugo ('40) developed a method for hypophysectomizing the chick embryo on the second day of incubation by removing the anterior part of the head which contains the pituitary primordia (Rathke's pouch and infundibulum). Although Fugo reported that the adrenal glands were not visibly affected by the operation, a reinvestigation of the problem in the chick embryo by Case ('51, '52) indicated that the pituitary gland was essential for the normal development of the adrenal glands. The adrenal weights and the adrenal ascorbic acid content of chick embryos, hypophysectomized by partial decapitation, were below normal in the last half of the incubation period (Case, '52). Betz ('67) reported that the cortical cords of normal embryos, after 20 days of incubation, comprised 44% of the adrenal glands and those of chick embryos hypophysectomized

on the second day only 31%. In hypophysectomized embryos there is also a delay in the development of the adrenal cortical cords and a decrease in the adrenal free cholesterol (Adjovi, '70) and a reduction in the corticosteroid concentration of the allantoic fluid (Woods et al., '71).

Betz ('67) hypophysectomized chick embryos by partial decapitation on the second day of incubation and then transplanted adenohypophyses from ten day old donors to the chorioallantoic membrane of the hypophysectomized embryos when they were nine days old. His study demonstrated that most of the defects of the hypophysectomized embryos were due to the absence of the hormones of the pars distalis.

The enzyme 3β-hydroxysteroid:NAD oxidoreductase (3β-hydroxysteroid dehydrogenase or 3β-HSD) is known to be involved in one of the early stages of steroid hormone biosynthesis, namely the oxidation of Δ^5-3β-hydroxysteroids to Δ^4-3-ketosteroids and is found in all known steroid producing tissues (Baillie et al., '66). Wattenberg ('58) developed a histochemical method to determine the presence of 3β-HSD in cells and tissues, thus enabling one to identify cells with a steroidogenic potential. Levy, Deane and Rubin ('58) using a modification of Wattenberg's method, reported that 3β-HSD activity in the rat adrenal was influenced by the pituitary. From the third day after hypophysectomy, there was a gradual decrease in 3β-HSD activity and by 50 days almost all activity had disappeared. When adrenocorticotropic hormone (ACTH) was injected into rats which had been hypophysectomized 70 days earlier, the atrophied adrenals began to hypertrophy and after eight days of treatment there was a restoration of the 3β-HSD activity in the inner zones.

Straznicky, Hajos and Bohus ('66) made a biochemical analysis of the adrenal glands of chick embryos and first observed 3β-HSD activity in 14 to 15 day old embryos. No 3β-HSD activity was observed in the adrenals of 17 day old hypophysectomized embryos. Manelli ('64), on the other hand, observed that histochemically demonstrable 3β-HSD activity of normal and hypophysectomized embryos appeared to be almost identical at 12 to 15 days and thus concluded that the absence of the pituitary does not influence the functional differentiation of the adrenal glands prior to 15 days of incubation. The results of preliminary work in our laboratory had shown that hypophysectomy has an effect on histochemically demonstrable 3β-HSD activity by nine days of incubation.

The purpose of this investigation was to study the activity, time of appearance and distribution of 3β-HSD in the adrenal glands of normal embryos, and embryos hypophysectomized prior to the onset of adrenal development, and to study the effects of adenohypophyseal transplants and exogenous ACTH on the activity of this enzyme in hypophysectomized embryos. A study of this nature should provide further information on the pituitary-adrenal interrelationships in embryos of the domestic fowl.

REVIEW OF THE LITERATURE

Histology and Development of the Avain Adrenal Gland

In the chick, the adrenal glands are paired encapsulated organs lying dorsal to the anterior end of the gonads and metanephric kidneys and just posterior to the lungs. As in mammals, the avian adrenal gland consists of two components, namely the interrenal tissue and the chromaffin tissue. In mammals the adrenal glands are divided into an outer cortex consisting of interrenal tissue and an inner medulla of chromaffin tissue.

In the domestic fowl the arrangement of the interrenal and chromaffin tissues differ from that of mammals. The interrenal cells are distributed throughout the gland in the form of irregularly arranged, frequently anastomosing columns interspersed with groups of chromaffin cells without distinct cortical or medullary zones. The terms "cortex" and "medulla" as applied to the avian adrenal gland refer only to the interrenal and chromaffin tissues, respectively.

The interrenal or cortical tissue is organized into solid cylindrical cords which appear in longitudinal section as double rows of columnar cells with long axes lying perpendicular to the cord (Sivaram, '65). In cross section, the cortical cord appears circular to oval and is composed of six or seven radially arranged cells. The end of each cord cell furthest from the center of the cord rests on a basement membrane and faces a blood sinus. The nucleus of each cell is situated away from the basement membrane and towards the center of the cord. In a longitudinal section of the cord, the nuclei are arranged in two rows while in cross sections they form a ring-

like pattern. The cortical cords in the peripheral region of the gland loop against the outer connective tissue capsule or the subcapsular layer of medullary cells. These loops are arranged radially around the periphery of the gland and delimit a superficial zone of cortical cords from a larger central zone (Sivaram, '65). The arrangement of cells into a double row in longitudinal sections of the cortical cords is clearly seen in the peripheral zone but is not so well defined in the central zone because the cords, which here are smaller in diameter, appear to twist, intertwine and anastomose. In the peripheral zone, the cortical cells are larger, oval or round in shape, and have considerable cytoplasm, and nuclei with diffuse chromatin. Pyknotic nuclei are rare in this zone (Kar, '47). The cortical cells of the central zone are elongate and smaller than those of the peripheral zone with smaller nuclei, many of which show various stages of pyknosis (Kar, '47).

The medulla is composed of closely packed chromaffin cells which form clumps and irregular masses among the cortical cords of the central zone (Sivaram, '65). A narrow layer of chromaffin cells is found between the connective tissue capsule and the peripheral cortical cords. The peripheral cortical cords are in contact with the capsule in a few places. The strands of chromaffin tissue which separate the large masses of cortical cords in the peripheral zone are, in general, smaller than those which separate the cortical cords in the central zone (Sivaram, '65).

The adrenal glands are highly vascular glands with numerous capillaries and blood sinuses ramifying around the cortical and medullary tissue. The blood sinuses commonly found in the peripheral zone are narrower than those of the central zone.

The cortical and medullary components of the adrenal are anatomically and embryologically separate structures. The cortical component arises from mesoderm and the medullary from ectoderm. Ericson ('68) studied the development of the adrenal gland of staged chick embryos during the first week of incubation. Other investigators (Hays, '14; Romanoff, '60; Sivaram, '65; and others) have also studied the development of the chick adrenal but they determined the age of embryos by the length of the incubation period. By employing the appearance of morphological characteristics (Hamburger and Hamilton, '51), specific events in adrenal morphogenesis can be pin-pointed to a specific stage of embryonic development rather than to a period of time. Since chick embryos during the first week of incubation are known to show considerable variation in development, the use of morphological characteristics to determine age is preferable and is now widely accepted.

In stage 21 (approximately 3.5 days incubation) embryos, at the level of the origin of the omphalomesenteric artery, a thickening of the peritoneal epithelium is seen lateral to the mesentery and usually immediately ventral to the subcardinal veins. A groove is generally present in the thickened peritoneum. Cells which eventually form the adrenal cortex are proliferated from this thickened peritoneal epithelium and may be seen in the mesenchyme immediately dorsal to the epithelium. These cells are round in shape, larger than the peritoneal cells, and their cytoplasm and nuclei stain lighter than those of the peritoneum.

By stage 22 (3.5 to 4 days), some of the proliferated cells have begun to migrate dorsally to a point in the mesenchyme dorsal to the subcardinal vein and between the dorsal aorta and the mesonephros. The number of

proliferated cells continues to increase, so that by stage 23 (4 days) a chain of cortical cells is seen lateral to the subcardinal vein extending from the groove in the peritoneal epithelium to a point in the mesenchyme, between the dorsal aorta and the mesonephros, where they form scattered groups composed of two or three cells.

The chromaffin tissue arises from cells migrating from the sympathetic trunks which in turn have originated from the neural crest (Hamilton, '52). At stage 23 large, oval, deep staining cells from the sympathetic trunks can be seen migrating ventrally in the mesenchyme between the aorta and the mesonephros, medial to the cortical cells.

In stage 24 (4.5 days) embryos, a connection between the cortical cells in the mesenchyme and the peritoneal epithelium is still evident. The number of cells in the cortical cell groups has increased at this stage. By stage 25 (4.5 to 5 days), there is no longer any connection between the cortical cells in the mesenchyme and the peritoneal epithelium, and the cortical cell groups now contain as many as eight or nine cells.

The cortical cell groups of stage 26 (5 days) embryos now contain more cells than those of previous stages and these are situated medial to the mesonephros and ventrolateral to the aorta. The change in position with respect to the mesonephros is due to growth of the mesonephros in a ventral direction. Most of the sympathoblasts migrating in the mesenchyme, between the aorta and the medial side of the cortical cell mass, continue to migrate to the ventral side of the aorta to form the prevertebral sympathetic plexuses; however, some of these migrating cells in these embryos become attached to the medial and dorsal sides of the cortical mass.

The cortical cell groups of stage 27 (5 to 5.5 days) embryos have now formed bodies in the mesenchyme between the aorta and the mesonephros and extend anteriorly to the posterior tip of the lung and posteriorly to the anastomosis of the subcardinal veins. Chromaffin cells, in addition to being on the medial surface of the cortical mass, are now located within this mass between the cortical cell groups. There is no apparent morphological difference between the cells which penetrate the cortical mass and those which continue to migrate to form the prevertebral sympathetic plexuses (Romanoff, '60). After penetrating the cortical mass, the chromaffin cells change from large circular cells with round, clear nuclei to irregularly shaped, small cells with oval nuclei (Hays, '14; Romanoff, '60). They are easily distinguished from the cortical cells by their strong affinity for basic stains. Some of the sympathoblasts also differentiate into neurons, forming a network of nervous fibers connecting the irregular cords of medullary cells with the outlying sympathetic trunks.

The first indications of the formation of a connective tissue capsule may be seen in stage 28 (5.5 to 6 days) embryos. Many of the chromaffin cells within the cortical mass are arranged in groups of two or three.

At stage 29 (6 to 6.5 days), the cortical cells become arranged into cords which in cross section are circular to oval, and contain about six or seven radially arranged cells. By stage 30 (6.5 to 7 days) the number of cells seen in cross sections of cords has increased to about 10 to 12 cells. The presence of blood cells in the spaces between the cords signifies the beginning of vascularization. The chromaffin cells at this stage of development tend to arrange themselves in cords throughout the

cortical cell mass, though many solitary cells may also be found (Hays, '14). The height of the migration of cells from the sympathetic trunks occurs at seven days and at this time the mesenchyme around the glands is filled with these cells.

In the eight day old embryo, a significant increase in the number of chromaffin cells is seen within the glands and only a few sympathetic cells remain in the mesenchyme. At this time the chromaffin cells are arranged in cords, the capsule is formed, and the invasion of the cortical mass by the sympathoblasts is complete(Sivaram, '65). The gland has now also become much more vascular.

The arrangement of the chromaffin cells undergoes a marked change in the nine day old embryo (Hays, '14). The chromaffin cords break down and the cells are organized into small groups.

After nine days of incubation the characteristic features of the adrenal gland have become firmly established and the development of the adrenal from this time to the time of hatching (21 days) is primarily one of the growth of the cortical and chromaffin tissues (Hays, '14). The glands increase in volume and vascularity. The cortical cells are arranged in irregular cords which pass around the blood sinuses and seem to form a foundation for the various components of the gland. The chromaffin cells, on the other hand, have no regular arrangement and are found in groups varying from two or three, to 30 or 40 cells each (Hays, '14). Each group is in contact with a blood sinus. By the seventeenth day of incubation the connective tissue capsule has become very dense (Hays, '14).

Adrenal-Pituitary Interrelationships in the Chick Embryo

The exact time during embryological development at which the chick adrenal becomes functional is not known. Straznicky and coworkers ('66) reported that in vitro adrenal tissue from 16 day old embryos had the potential for synthesizing progesterone. The adrenals of 14 day old embryos in vitro were found to synthesize corticosterone, 11-deoxycorticosterone, and aldosterone (Bonhommet and Weniger, '67). Pedenera ('71) used a bioassay method to detect the secretion of corticosteroids in eight day old embryos. Histochemically demonstrable lipids and cholesterol have been reported in five day old embryos (Dawson, '53; Castañé Decoud et al., '64).

The presence of 3β-hydroxysteroid:NAD oxidoreductase (3β-hydroxysteroid dehydrogenase or 3β-HSD in a tissue, an enzyme involved in one of the initial steps of steroid hormone biosynthesis, has been regarded as an indication of such synthesis. Boucek, Gyori and Alvarez ('66) first observed the presence of this enzyme in the adrenocortical cells of five day old embryos, Chieffi, Manelli, Botte and Mastrolia ('64) at 4.5 days, and Ericson and Domm ('69) at 4 days (stage 23). However, it must be noted that the presence of the emzyme is only indicative of steroidogenic activity since the presence of a single emzyme in the biosynthetic pathway does not necessarily imply successful hormone synthesis (Lobel et al., '62).

The exact time at which the anterior pituitary begins to exert its adrenocorticotropic influence in the chick embryo is also not known. Szekely and coworkers ('58) used a bioassay method to detect ACTH activity in extracts of pituitary gland from chick embryos of eight days and

observed a sudden increase in the synthesis or release of this hormone on the 12th day. At eight days, Toth, Simon and Szekely ('58) observed an increase in mitotic activity in the adrenocortical cells of the chick embryo which by ten days had returned to its original level. Embryos which were hypophysectomized by partial decapitation on the second day of incubation did not show this increase in the mitotic activity of these cells during the eighth day. From these observations it was concluded that histological differentiation of the adrenal cortex in the chick embryo is dependent on the anterior pituitary as early as the eighth day. However, Castañé Decoud, Pedernera and Narbaitz ('64) maintain that the mitotic index may not be an adequate measure of the functional activity of the adrenal cortex.

A number of investigators have reported that the chick adenohypophysis exerts its adrenocorticotropic effect during the last half of embryonic development. Case ('52) hypophysectomized white Leghorn chick embryos at stages 11 to 13 by the partial decapitation method of Fugo ('40) and studied the resulting effects on adrenal weight and ascorbic acid content. He found that the adrenal weights of normal and hypophysectomized embryos were about the same in 14 and 15 day old embryos, however, after 16 days of incubation the adrenals of normal embryos showed a steady increase while those of hypophysectomized embryos remained at about the weight observed in 15 day old embryos. The adrenal ascorbic acid content of 15 to 19 day old hypophysectomized embryos remained at the level observed in normal 12 and 13 day embryos. These results led this investigator to assume that the ascorbic acid values of 12 and 13 day old normal chick embryos represent a minimal value attained in the absence of anterior pituitary stimulation and that this value may

represent medullary ascorbic acid, since the medulla revealed characteristically high concentrations of ascorbic acid early in embryonic life. From this it was concluded that the adrenal ascorbic acid mobilizing effects of the anterior pituitary came into play on about the 13th day.

The differentiation of the duodenal epithelium has been used as an indicator of adrenal cortical secretion (Moog, '59). Chick embryos injected with cortisone acetate or hydrocortisone acetate showed an acceleration in the rate of differentiation of the duodenal epithelium and a precocious production of alkaline phosphatase (Moog and Richardson, '55; Moog and Thomas, '57). The same effects were brought about by the administration of ACTH (Moog and Ford, '57; Moog, '59). Watterson, Brown and Bartha ('59) did not observe alkaline phosphatase in the duodenal epithelium of most of their hypophysectomized 16 to 19 day old chick embryos. In hypophysectomized embryos where this enzyme occurred, the amount observed was always less than that of normal 17 day old embryos. These investigators concluded that their results indicate an ACTH control of the adrenal cortex by 16 days of incubation. The effects of hypophysectomy by partial decapitation, on the morphological and chemical differentiation of the duodenal epithelial cells, were first encountered in 14 day old white Leghorn chick embryos (Hinni and Watterson, '63). The differentiation of these cells was arrested at levels characteristic of 17 to 18 day old embryos. Thus, it would appear that under normal circumstances growth and differentiation of the epithelial cells covering the duodenal villi is stimulated by adrenal cortical hormones in response to ACTH. However, Moog ('61) reported that the functional differentiation of the duodenum of chick embryos is affected by the status

of the thyroid gland. She considered thyroxine to be permissive in conditioning the sensitivity of the duodenum to adrenocorticoids by providing the necessary condition in which cortisone can exert its positive effect. Hinni and Watterson ('63) reported that cortisone and thyroxine either alone or together, did not alleviate the arrested duodenal differentiation of hypophysectomized chick embryos and suggest that somatotropic hormone, which is essential in maintaining the normal turnover rate of the duodenal mucosa in rats, may also be essential for normal duodenal differentiation in chick embryos. Thus, the possibility exists that the endocrine control of growth and differentiation, of duodenal epithelial cells in normal chick embryos, is relatively complex and, therefore, does not actually reflect the functional status of just the adrenal glands.

Adrenal Steroidogenesis

Sandor ('69) reported that the avian adrenal glands synthesize almost exclusively 17-deoxycorticosteroids: 11β,21-dihydroxypregn-4-ene-3, 20-dione (corticosterone), 11β,18,21-trihydroxypregn-4-ene-3, 20-dione (18-hydroxycorticosterone and 11β, 21-dihydroxy-3,20-dioxopregn-4en-18-al (aldosterone). An in vitro study of steroidogenesis in the adrenal glands of four avian species, including juvenile chickens, was made by deRoos ('61) who identified corticosterone and aldosterone as the major hormones synthesized from endogenous precursors. These studies were confirmed by Donaldson and coworkers ('65) who not only identified corticosterone and aldosterone but also 18-hydroxycorticosterone, as products of avian adrenal tissues incubated in vitro. In vitro studies of steroidogenesis in the avian adrenal have

shown sodium acetate, cholesterol, 3β-hydroxypregn-5-en-20-one (pregnenolone), pregn-4-ene-3, 20-dione (progesterone) and 21-hydroxypregn-4-ene-3, 20-dione (11-deoxycorticosterone) to be precursors of corticosterone, aldosterone, and 18-hydroxycorticosterone (Sandor and Lanthier, '63; Sandor et al., '63, '65; Hall and Koritz, '66; Sandor, '69). Although the pathways of steroidogenesis in the avian adrenal have not been completely worked out, these in vitro studies have shown great similarities to the pathway established for the zona glomerulosa of the mammalian adrenal glands and seem to conform to the sequence--acetate--cholesterol--pregnenolone--progesterone--11-deoxycorticosterone--corticosterone--18-hydroxycorticosterone and aldosterone (Sandor, '69).

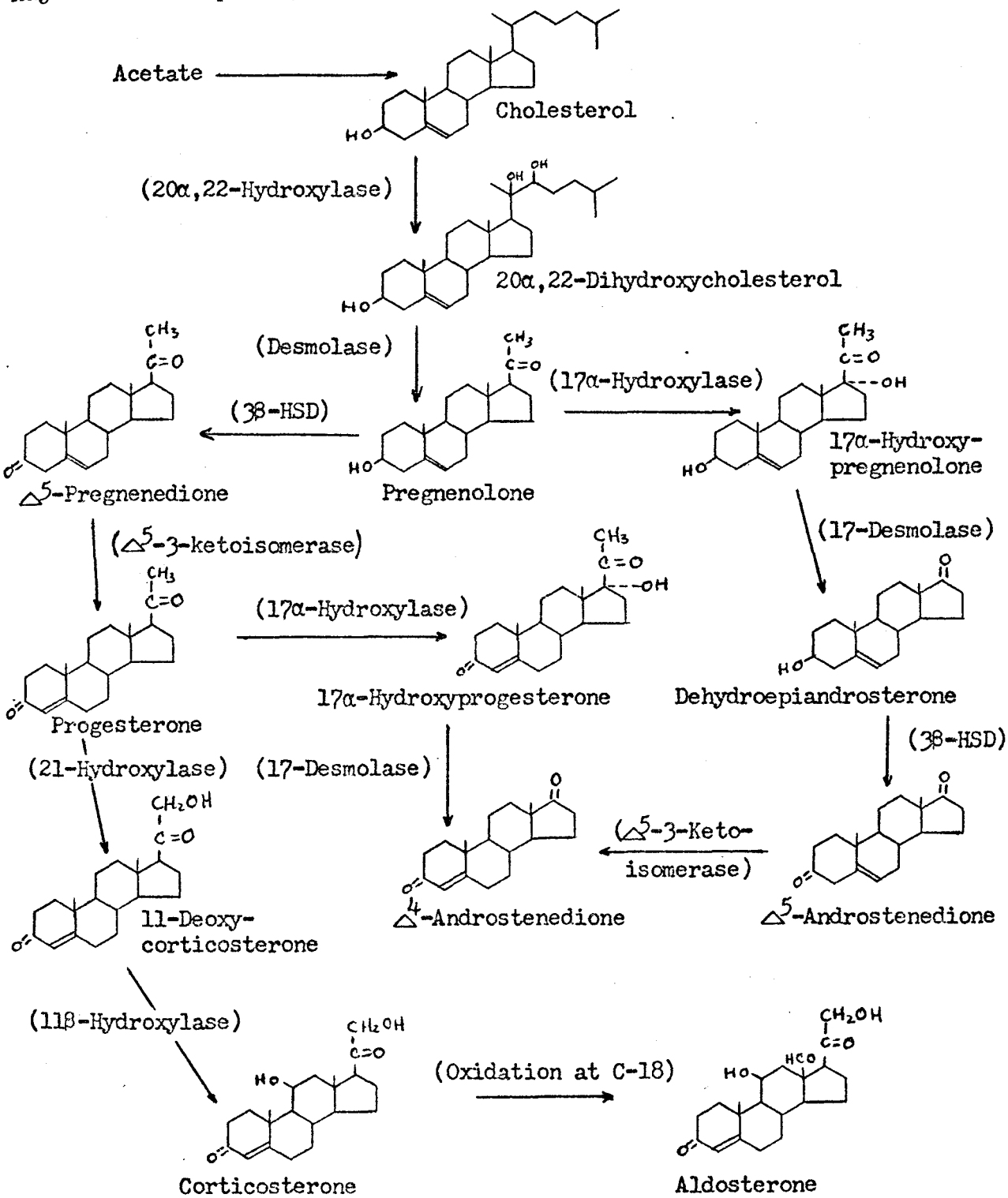
In addition to the steroids with functions characteristic of the adrenal cortex, androgenic steroids have been isolated from mammalian adrenal glands (Short, '60) and also have been detected in the adrenals of chickens (McGowen, '36; Arrington, et al., '52). McGowen ('36) observed virilism in a hen with an adrenocortical tumor and Arrington and coworkers ('52) have shown that adrenal glands from young chicks, implanted into an incision in the base of the comb of three day old chicks, produced an effect only slightly inferior to that of testicular tissue. Roberts and Szego ('55) reported that the mammalian adrenal cortex secretes androst-4-ene-3, 17-dione (Δ^4androstenedione), 11β-hydroxy-androst-4-ene-3,17-dione (11β-hydroxy-androstenedione) and androst-4-ene-3,11,17-trione (andenosterone). The in vitro production of these androgens from acetate has been demonstrated in slices of human adrenal (Bloch, Dorfman and Pincus, '56; Bloch, Pincus and Dorfman, '56). In the adrenal glands Δ^4-androstenedione is largely

derived from 3β-hydroxyandrost-5-en-17-one (dehydroepiandrosterone or DHA) which has been identified in human fetal adrenal tissue (Bloch, Benirschke and Rosemberg, '56).

Assuming that the biosynthetic pathways of adrenal corticosteroids in birds are similar to those of mammals, the diagram on page 16 would indicate the metabolic pathways involved in the biogenesis of these steroids. The following are the systemic names for the trivial terms used in the following diagram:

Trivial Name	Systematic Name
aldosterone	11β,21-dihydroxy-3,20-dioxopregn-4-en-18-al
Δ^4-androstenedione	androst-4-ene-3,17-dione
Δ^5-androstenedione	androst-5-ene-3,17-dione
corticosterone	11β-21-dihydroxypregn-4-ene-3,20-dione
dehydroepiandrosterone	3β-hydroxyandrost-5-en-17-one
11-deoxycorticosterone	21-hydroxypregn-4-ene-3,20-dione
3β-HSD (3β-hydroxysteroid dehydrogenase	3β-hydroxysteroid:NAD oxidoreductase
17α-hydroxypregnenolone	3β-17α-dihydroxypregn-5-en-20-one
17α-hydroxyprogesterone	17α-hydroxypregn-4-ene-3,30-dione
Δ^5-3-ketoisomerase	3-ketosteroid Δ^4- Δ^5-isomerase
Δ^5-pregnenedione	pregn-5-ene-3,20-dione
pregnenolone	3β-hydroxypregn-5-en-20-one
progesterone	pregn-4-ene-3,20-dione

Major metabolic pathways involved in biogenesis of adrenocortical steroids.



For systematic names, see page 15.

(Dorfman and Sharma, '65; Samuels and Eik-Nes, '68; Samuels and Uckekawa, '67)

Histochemistry of the Embryonic Avian Adrenal Gland

According to Deane ('62) the characteristic chemical properties of adrenocortical interrenal cells which distinguish them from most other types of cells, are: (1) a high lipid content with a large fraction consisting of cholesterol esters; (2) a high concentration of ascorbic acid; and (3) the presence of enzymes that take part in the synthesis of steroids and the specific cortical hormones.

Dawson ('53) used a group of histochemical reactions, characteristic of the lipid droplets of steroid producing organs, to determine the time at which histochemical differentiation of the adrenocortical cells of chick embryos occurred. These lipid droplets react positively in tests for carbonyl groups (Schiff test and naphthoic acid hydrazine method of Ashbell and Seligman, '49) as well as staining with Sudan black B and osmic acid. When cholesterol or its esters are present in the lipid droplets, a blue-green color results following exposure of tissue sections to concentrated sulfuric acid and glacial acetic acid (Schultz, '24). Cholesterol and its esters display a birefringence when viewed with a polarizing microscope (Lillie, '66). Dawson ('53) observed positive histochemical responses (osmiophilia, sudanophilia, Schiff reaction, Schultz reaction and birefringence) in cortical tissue of embryos as early as six days and seven hours incubation. Using the Ashbell-Seligman ('49) method, Dawson observed carbonyl groups in nine day old chick embryos, the youngest embryos studied by this method. In the early stages of cortical development, positive reactions appeared to be confined to cortical tissue that had become arranged into definite cords. Positive histochemical reactions were not uniformly and consistently present

until 11 to 12 days of incubation at which time ascorbic acid also appeared.

Castañé Decoud, Pedernera and Narbaitz ('64) observed the presence of sudanophilia in the adrenocortical cells of five day old chick embryos and birefringence in six day old embryos. Narbaitz and Sabatini ('63) reported the presence of cholesterol in the adrenal glands of seven day old chick embryos, the youngest embryos used in their investigation.

Sivaram ('69) made a histochemical study on the appearance and distribution of various types of lipids in the adrenal cortex of chick embryos and chicks up to one week after hatching. The concentration of the lipids was based on an evaluation of the staining intensities in arbitrary units of one to ten. The oil red 0 method was employed in determining the presence of glycerides plus long chain fatty acids in the adrenal cortex of chick embryos beginning at seven days of incubation. The relative concentration of lipid droplets increased steadily from seven days through hatching with a noticeable increase in the 12 day embryo. In the first week after hatching the increase in concentration was somewhat slower than during the embryonic period. With the nile blue method, phospholipids stain dark blue and were first present in embryos at six days of age while the neutral lipids stain pink and were first detected after nine days of incubation. The concentration of the phospholipids increased rapidly from seven to 11 days and then somewhat more slowly until they reached the maximum level just before hatching. A level slightly less than the maximum was maintained in the post embryonic period. Neutral lipids increased steadily in concentration through the latter half of the incubation period and continued to do so, though at a slower rate, after hatching. Neutral lipids were first

observed in the cords of the peripheral zone at the time of hatching. Unsaturated lipids were detected with the U.V.-Schiff reaction in the adrenal cortical cells beginning with the seven day old embryo. The intensity of this reaction increased with the age of the embryo and the chick. Cholesterol was first observed with the Schultz reaction in the adrenal cortex of 10 day old embryos and its concentration increased through the remainder of the embryonic and the first week of the postembryonic period.

With the use of a histochemical method, Dawson ('53) observed that ascorbic acid first appeared in the adrenal cortex of the nine day old chick embryo but did not occur in appreciable quantities until after 11 days of incubation. Sivaram ('68) employed a modification of the histochemical method used by Dawson for the detection of ascorbic acid and observed that adrenal ascorbic acid first appeared in the ten day old chick embryo. There was a fairly rapid rise in staining intensity and the adult level was almost reached prior to hatching. The peripheral cortical zone frequently exhibited a greater concentration of ascorbic acid than the central zone.

Case ('51) used a biochemical assay to determine the adrenal ascorbic acid content of normal embryos from 12 days through hatching and in chicks up to nine days after hatching. Adrenal ascorbic acid was found to increase gradually from 0.85 mg/gm in the 12 day embryo to a maximum of 1.75 mg/gm at 7 days, after which time there was a gradual decline which continued through hatching to 1.30 mg/gm in the four and nine day old chick.

According to Deane ('62) the enzymes found in adrenocortical tissues fall into three classes: (1) those involved directly in the biosynthesis of steroid hormones; (2) those found generally in active cells; and (3) those

found variably in adrenocortical and other kinds of cells.

The first category includes the enzymes involved in steroid synthesis from acetate and the dehydrogenases and hydroxylases involved in specific conversions of precursors to adrenocortical hormones. Wattenberg ('58) developed a histochemical method to reveal the presence of the enzyme reaction in which pregnenolone was converted to progesterone or dehydroepiandrosterone (DHA) to Δ^4-androstenedione. Two enzymes, 3β-hydroxysteroid:NAD oxidoreductase (3β-hydroxysteroid dehydrogenase or 3β-HSD) and 3-ketosteroid Δ^4-Δ^5-isomerase, are involved in this transformation. 3β-HSD oxidized the 3β-hydroxyl group and required nicotinamide adenine dinucleotide (NAD), and 3-ketosteroid Δ^4-Δ^5-isomerase catalyzed the migration of the double bond from the 5-6 position to the 4-5 position (Samuels and Uchikawa, '67). Bongiovanni and Root ('64) reported that all hormonally active steroids are synthesized biologically by pathways involving such a synthetic step, and that 3β-HSD activity is essential in steroidogenic endocrine tissues in the early biosynthesis of these hormones. There is at present no general agreement as to the time when development of 3β-HSD activity first occurs in the adrenal glands of chick embryos. Straznicky, Hajos and Bohus ('66) assayed adrenal homogenates for 3β-HSD and first observed this enzyme in 14-15 day old chick embryos. Using histochemical methods, Sivaram ('64) first observed the enzyme in ten day embryos, Boucek, Gyori and Alvarez ('66) at five days, Chieffi, Manelli, Botte and Mastrolia ('64) at 4.5 days and Ericson and Domm ('69) at four days (Hamburger-Hamilton stage 23).

The second category includes oxidative enzymes. Mastrolia and Manelli ('67) used histochemical methods to locate glucose-6-phosphate

dehydrogenase and 6-phosphogluconate dehydrogenase in the adrenal glands of chick embryos. The reactions for these enzymes were first observed in the adrenal glands of chick embryos after 4.5 days of incubation and were found to increase in intensity throughout the remainder of the incubation period. Sivaram ('65) stained sections of adrenal glands of chick embryos for succinate dehydrogenase to mark the mitochondria in order to study their distribution. She did not report the time at which succinate dehydrogenase first occurred but from the 18th day of incubation the cells in the peripheral cortical zone showed a greater abundance of mitochondria than those of the central zone.

The third category included hydrolytic enzymes. Sivaram ('68) employed histochemical methods to study aliesterases (non-specific carboxylic esterases) and non-specific cholinesterases in the adrenal cortex of embryonic, juvenile, and adult chickens. Non-specific esterases capable of hydrolyzing α-naphthyl acetate and naphthol AS-acetate were abundant in the cortical cells during embryonic and post-embryonic periods. They were first detected in embryos after six days of incubation. Their concentration increased steadily with time until a maximum was attained at the time of hatching. This concentration was maintained for a few days after hatching and then decreased to a low level which was sustained in the adult. Beginning on the 15th day of incubation and continuing throughout life, the cortical cords in the peripheral zone revealed a higher concentration of this enzyme than the cords of the central zone. Sivaram ('68) reported a small amount of cholinesterase in the adrenal

cortex of chick embryos. This enzyme was first detected in the cortical cells after nine days of incubation. Beginning on the 15th day, the enzymatic activity was slightly greater in the cortical cords of the peripheral than in those of the central zone. In the final week of the embryonic period, there was a slight increase in cholinesterase which reached a maximum at the time of hatching and remained at this level during later life. The maximum cholinesterase activity in the cortical cells was not higher than that of the medullary cells when they first appeared.

Hypophysectomy in Chick Embryos

In 1940, Fugo reported a method for eliminating the pituitary gland in chick embryos by removing the pituitary primordia. A small opening was cut through the shell directly over an embryo which had been incubated for 33 to 38 hours. The vitelline membrane was slit and a transverse cut was made at the level of the mesencephalon. The anterior part of the head containing the pituitary primordia (Rathke's pouch and infundibulum) was then completely removed, the egg sealed and returned to the incubator. These partially decapitated embryos, which in addition to lacking both rudiments of the pituitary, also lack eyes, upper beak, and all other derivatives of the prosencephalon, and are called 'hypophysectomized' embryos. The results of Fugo's experiments indicated that although the embryonic pituitary had no effect on primary morphological differentiation it did become active during the second half of the incubation period. Retarded

somatic growth and defective differentiation of the gonads and thyroid were observed in these embryos, however, the adrenal glands were not visibly affected by this operation.

A reinvestigation of the adrenal-pituitary interrelationships in chick embryos by Case ('51, '52) indicated that the pituitary gland was essential for normal development of the adrenal glands. Chick embryos were hypophysectomized by the partial decapitation method of Fugo ('40) at stages 11 to 13 (approximately 40 to 52 hours incubation). The adrenal weights of normal and hypophysectomized embryos were about the same on the 14th and 15th day; however, after the 16th day those of normal embryos increased steadily while those of hypophysectomized embryos remained at about the weight observed on the 15th day. The adrenal ascorbic acid content of 15 to 19 day old hypophysectomized embryos remained at the level observed in normal 12 and 13 day old embryos. Case ('52) also observed that the volume of the lipid-containing cortical tissue (paper weight method) in the adrenal glands of hypophysectomized embryos of 18 days was below normal.

Betz ('67) reported that in the adrenal glands of normal 20 day old chick embryos the cortical cords, chromaffin tissue and non-parenchymal space comprise 44, 33, and 23% of the glands while in 20 day old embryos hypophysectomized by Fugo's ('40) method the percentages were 31, 40, and 29.

Adjovi ('70) decapitated chick embryos after from 8 to 13 days of incubation and examined the adrenal glands two to four days later. She found that decapitation caused some delay in the development of the cortical

cords and a drop in the free cholesterol content.

Straznicky, Hajos and Bohus ('66) hypophysectomized chick embryos by decapitation on the second day of incubation and observed that the ultrastructural differentiation of the adrenal glands was retarded in embryos examined on the 14th to 18th day. The effects of hypophysectomy were most striking in the mitochrondria which were very similar to those observed in 6 to 8 day old embryos. These investigators also determined 3β-hydroxysteroid NAD oxidoreductase (3β-hydroxysteroid dehydrogenase or 3β-HSD) and 3-keto-steroid Δ^4-Δ^5-isomerase activity by assaying the formation of androst-4-ene-3, 17-dione (Δ^4-androstenedione) from exogenous 3β-hydroxyandrost-5-en-17-one (dehydroepiandrosterone or DHA). Enzyme activity was first detected with DHA in 14 and 15 day old embryos with a peak in activity occurring at about the time of hatching (21 days). No enzyme activity was detected in the adrenal glands of normal embryos 9 to 12 days of age or in 17 day old hypophysectomized embryos.

Manelli ('64) used a histochemical method to study 3β-HSD activity in the adrenal glands of chick embryos hypophysectomized by Fugo's ('40) method and necropsied after 12 to 15 days of incubation. No differences were observed between hypophysectomized and control embryos up to 15 days. On the basis of this evidence the author concluded that the absence of the pituitary gland does not influence the functional differentiation of the adrenal glands in chick embryos prior to 15 days of incubation.

Pituitary Chorioallantoic Grafts in Chick Embryos

In the chick embryo, the chorioallantoic membrane is formed when the mesodermic layer of the allantois fuses with the adjacent mesodermic layer of the chorion beginning on the fifth day of incubation. This double layer of mesoderm develops an extremely rich vascular network which is connected with the circulation of the embryo by the allantoic arteries and veins. It is through this circulation that the allantois carries on its primary function of oxygenating the blood of the embryo and relieving it of carbon dioxide. This is made possible by the position of the allantois, immediately beneath the porous shell. The highly vascularized chorioallantoic membrane is capable of supporting the growth of living tissues (grafts) placed in contact with it (Rawles, '52). Capillaries grow very rapidly into such grafts and vascular connections are often established within 24 hours (Minoura, '21). The optimum time for grafting tissues onto this membrane is from the eighth to the eleventh day of incubation (Willier, '24) since during this period the capillary network of the chorioallantoic membrane is approaching the surface of the membrane.

Since chorioallantoic grafts are not in actual contact with the body of the host embryo, the probability of inductive reactions is minimized; however, the vascularization of the grafts is connected with the circulation of the host and they are therefore exposed to hormones and other substances that may be carried in the blood stream (Rawles, '52).

Betz ('67) studied the effects of chorioallantoic, pars distalis grafts on the development of chick embryos hypophysectomized at stage 10 or

11 by the partial decapitation method of Fugo ('40). After nine days of incubation pars distalis grafts from ten day old donors were placed on the chorioallantoic membrane of hypophysectomized embryos. The embryos were necropsied after 20 days of incubation and the grafts, gonads, thyroid, and adrenal glands examined. Histological examination of the grafts revealed normal cells. The amount of pars distalis parenchyma in embryos with one graft was 88% of normal and in those with two grafts it was 155% of normal. The average weight of normal paired adrenal glands was 4.5 mg while that of hypophysectomized embryos was only 2.6 mg. The average paired adrenal weight of embryos with pars distalis grafts was 3.7 mg. In the adrenal glands of normal embryos the cortical cords, chromaffin tissue and non-parenchymal space comprised 44, 33, and 23% of the photographed fields. In hypophysectomized embryos the percentages were 31, 40, and 29 while in embryos with pars distalis grafts they were 43, 31, and 26 respectively. It was concluded from these observations, along with those on the gonads, thyroids and body weight, that most of the defects seen in hypophysectomized embryos were due to the absence of the hormones of the pars distalis, and that the grafts of the pars distalis secreted normal amounts of adrenocorticotropic, gonadotropic, thyrotropic and somatotropic hormones.

Woods and Weeks ('69) studied 3β-HSD activity in the gonads of normal and hypophysectomized chick embryos and chick embryos receiving chorioallantoic pituitary grafts. Beginning with 13.5 day old embryos, there was a noticeable reduction in 3β-HSD activity in sections of the testis and the ovarian cortex of hypophysectomized embryos when compared to that observed

in intact control embryos. Beginning with 16.5 day old embryos, there was less 3β-HSD activity in the medulla of the left ovaries of hypophysectomized embryos than in those of intact controls. Pituitary transplants on the chorioallantoic membrane of hypophysectomized embryos brought about an elevation in the 3β-HSD activity of gonads to levels characteristic of intact embryos. These observations were interpreted as indicating that the pituitary transplants stimulated the activity of this enzyme, and in consequence gonad steroid hormone synthesis, during the last half of the incubation period.

Effects of Exogenous ACTH

Adrenocorticotropic hormone (ACTH) is known to stimulate the growth and development of adrenal cortical tissues and the elaboration of adrenocortical hormones. The avian pituitary, like that of mammals contains ACTH or an ACTH-like substance (deRoos and deRoos, '64; Resko et al., '64). Chemically pure ACTH has been prepared from the pituitaries of a number of mammalian species, but little is known about the chemical nature of avian pituitary ACTH (Sturkie, '65). deRoos and deRoos ('64) incubated avian adrenal tissue, with mammalian ACTH and with acid extracts of chicken adenohypophyses, and observed a significant increase in the secretion of both corticosterone and aldosterone. Their results indicate that in non-treated controls aldosterone was produced in greater quantity than corticosterone but under the influence of mammalian ACTH, or chicken adenohypophyseal extracts, corticosterone was the major secretory product. The magnitude of

the response to ACTH, or adenohypophyseal extracts, was greater for corticosterone than for aldosterone. Since the steroidogenic response of the chicken adrenal gland to the two hormone preparations was essentially the same, the authors concluded that both hormones possess similar biological properties. These workers also reported that acid extracts from the pituitaries of several chondrichthyean fish stimulated corticoid production by the chicken adrenal gland *in vitro* and they, therefore, concluded that there may be an absence of species specificity in the ACTH of pituitaries obtained from different vertebrate species. They were unable to determine the degree of ACTH activity in the chicken pituitary, however, their approximations were similar to the average yields reported by Fortier ('59) who used a similar acid extraction procedure in the rat. Resko and coworkers ('64) injected chicken pituitary extracts in cockerels at 25 weeks of age and observed a three-fold increase in corticosterone concentrations in the adrenal venous plasma.

Since no chemically pure avian ACTH has thus far become available, mammalian ACTH has been used in experimental work involving the effects of exogenous ACTH in the bird. The administration of mammalian ACTH in chickens has resulted in an increase in adrenal weights (Zarrow *et al.*, '62), increased adrenal corticosterone production (Taylor *et. al.*, '70) and changes in the ultra-structure of the adrenals which reflect a hyperactive state of the interrenal cells (Kjaerheim, '68). Mammalian ACTH has also been reported to have an effect on the adrenal glands of chick embryos *in vivo* and *in vitro*. Moog and Ford ('57) treated 11 day old chick embryos with ACTH and observed

that the adrenals were significantly heavier than those of saline-injected controls and that this difference was maintained up to 19 days, the last stage studied.

Manelli and Mastrolia ('63) reported the in vitro effects of ACTH on 3β-HSD activity in the adrenal glands of chick embryos. The adrenal glands of 12 day old embryos were cultured in media with and without ACTH. After six days of culturing, the 3β-HSD reaction was almost equal in ACTH treated and non-treated control adrenal glands. By seven days, it had decreased in control adrenals but remained almost unvaried in the ACTH treated. After ten days of culturing, the reaction for 3β-HSD in ACTH treated adrenal glands was definitely positive and was distributed throughout the glands while in non-treated controls the reaction was nearly absent or localized in a narrow zone along the periphery of the gland.

Several investigators have reported that the embryonic chicken adrenal gland is susceptible to ACTH stimulation in the early stages of differentiation. Castañé Decoud and coworkers ('64) administered daily injections of ACTH in chick embryos during the third and fourth days of incubation. The embryos were necropsied on the fifth and sixth day and the adrenals studied histochemically for lipids (Sudan black B) and for cholesterol and its esters (birefringence). Both sudanophilia, which appeared on the fifth day, and birefringence, which appeared on the sixth day, increased in frequency and intensity following ACTH treatment.

Pedernera ('68) incubated adrenal glands from six and ten day old chick embryos for 6 to 12 days in media with and without ACTH. After 6 and 12 days of culturing, the number of adrenal glands from 6 day

old embryos showing sudanophilic material and birefringent crystals had increased in media with ACTH and decreased in that without ACTH. After six days of culturing all of the adrenal glands of ten day old embryos, incubated in media with and without ACTH, contained sudanophilic and birefringent material. After 12 days, over 60% of the adrenals cultured in media without ACTH had lost their lipidic material while only a few of the adrenals cultured with ACTH had lost this material. These results indicated that the accumulation of lipids, in the embryonic chick adrenal glands maintained in vitro, was dependent on the presence of ACTH in the medium.

Pedernera ('71) also investigated the secretory capacity of the adrenal glands of chick embryos at different stages of development using a bioassay for corticosteroids based upon the in vitro modification produced by these hormones on the height of the chick embryo duodenal mucosa. He found that the adrenal of chick embryos have the potential to secrete corticosteroids beginning on the eighth day and as early as the fifth day when mammalian ACTH is added to the medium.

The investigations of Nagra, Birnie, Baum and Meyer ('63), Straznicky, Hajos and Bohus ('66), Adjovi ('70), and Case ('52) have shown that ACTH administration stimulates the adrenal glands of hypophysectomized chickens and chick embryos. A decrease in the corticosterone level of adrenal venous blood was observed in chickens hypophysectomized when 7.5 weeks of age (Nagra et al., '63). The intravenous administration of ACTH in such birds elevated the production of corticosterone. Straznicky, Hajos and Bohus ('66) observed a retardation in adrenal ultrastructural differentia-

tion in chick embryos hypophysectomized by decapitation. The administration of ACTH in such embryos counteracted the effect of hypophysectomy. Adjovi ('70) found that decapitation of chick embryos caused some delay in the development of the adrenal cortical cords and a drop in the adrenal free cholesterol content while the adinistration of ACTH in such embryos restored the normal development of cortical cords but had no effect on the level of free cholesterol. Case ('52) observed a decrease in the volume of lipid-containing cortical tissue (paper weight method) in the adrenal glands of 18 day old hypophysectomized chick embryos. The administration of ACTH in such embryos resulted in an increase in the volume of lipid containing tissue when compared with hypophysectomized controls. However, despite the apparent increase in cortical tissue no increase in the total size of the gland was evident.

MATERIALS AND METHODS

White Leghorn eggs from a commercial breeder[1] and brown Leghorn eggs from a flock maintained in the laboratory of Professor L. V. Domm and from a bird fancier[2] were used in this study. The eggs were incubated in a forced draft incubator maintained at a temperature of 37.8 ± 0.5°C and a humidity of 62 ± 2%. The chronological ages of the embryos given at time of necropsy, represent the actual time the eggs had been in the incubator. However, since there is a great deal of variation in the degree of development of embryos of the same chronological age, especially during the first week of incubation (Hamilton, '52), all embryos necropsied during the first week of incubation were staged according to the morphological characteristics established by Hamburger and Hamilton ('51).

The following experiments were performed: I. A study on the development and distribution of 3β-hydroxysteroid:NAD oxidoreductase (3β-hydroxysteroid dehydrogenase or 3β-HSD) activity, in the adrenal gland of normal chick embryos, throughout the entire incubation period and in the first two weeks after hatching. II. The effect of hypophysectomy on 3β-HSD activity in the chick adrenal gland during the embryonic period. III. The effect of adenohypophyseal chorioallantoic grafts on adrenal 3β-HSD activity in hypophysectomized chick embryos. IV. The effect of exogenous ACTH on adrenal 3β-HSD activity in hypophysectomized chick embryos.

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[1] Len Sharp Commercial Hatchery, Glen Ellyn, Illinois.

[2] Russell A. Stauffer, Rt. 1, Wooster, Ohio.

I. Adrenal 3β-HSD activity in normal chick embryos and chicks.

White and brown Leghorn embryos were necropsied at stages 21 through 32 (3.5 to 7.5 days incubation) and at 24 hour intervals from eight days through hatching. Some normally hatched chicks between the ages of one and 14 days were also necropsied. Embryos necropsied during the first week of incubation were staged according to the method of Hamburger and Hamilton ('51) while alive inside the shell. These embryos were then removed from the shell, decapitated and immediately quick frozen, in an extended position, on a freezing plate in an ultra deep freezer maintained at -78°C. Embryos older than seven days were removed from the shell, decapitated and the torso, minus wings and legs, quickly frozen. All chicks were decapitated and the torso trimmed before freezing, into a narrow block containing the adrenal glands attached to underlying tissues. All frozen tissues were wrapped in multiple layers of Saran Wrap and stored in sealed plastic vials at -78°C until they were sectioned and processed. Some embryos were fixed in Bouin's fluid, paraffin embedded, and sectioned and stained with hematoxylin and eosin in order to serve as morphological controls for comparison with non-stained frozen sections.

The frozen embryos were mounted on a microtome object disc and sectioned at 10μ (-20°C) on a cryostat microtome. Embryos of three to seven days were sectioned in toto while those eight days and older were trimmed, on the object disc, into a block which contained the adrenal glands and some adjacent tissues. Spaced serial sections were mounted on three coverslips by thawing. All sections were air dried for 15 minutes at room temperature in order to attach the sections to the coverslips.

The technique for the histochemical visualization of 3β-HSD activity employed in this investigation was a modification of Levy, Deane and Rubin's ('59) modification of Wattenberg's method ('58). Sections mounted on coverslips were placed in a 0.1M phosphate buffer, pH 7.2, for 15 minutes to remove endogenous substrates (Levy et al., '59). The sections on two of the coverslips (experimental sections) were then incubated for two hours at 38°C in Columbia jars containing the substrate media. 3β-Hydroxyandrost-5-en-17-one (dehydroepiandrosterone or DHA) and 3β-hydroxypregn-5-en-20-one (pregnenolone) were used as the substrates for all adrenals examined. 1,2-Propanediol (propylene glycol) was used to solubilize the substrate. The substrate was first dissolved in acetone and then 1.0 ml of acetone, containing 0.2 mg of substrate, was put in a dry Columbia jar and the acetone evaporated prior to the addition of propylene glycol and the other constituents. The medium used in this study was similar to that of Woods and Damm ('66) who omitted nicotinamide. Nicotinamide was in the medium of Levy, Deane and Rubin ('59). The composition of the substrate medium was as follows:

Constituent	Volume	Weight
Dehydroepiandrosterone[3]		
or		0.2 mg
Pregnenolone[3]		
Propylene glycol[4]	0.5 ml	
NAD[5] (3.0 mg/ml in distilled water)	0.8 ml	2.4 mg
Nitro-BT[3] (1.0 mg/ml in distilled water)	1.0 ml	1.0 mg
Phosphate Buffer, 0.1M, pH 7.2	4.7 ml	
Total Volume	7.0 ml	

[3] Mann Research Laboratories, New York, N.Y.
[4] Eastman Organic Chemicals, Rochester, N.Y.
[5] Sigma Chemical Company, St. Louis, Mo.

The sections on the third coverslip (substrate controls) were incubated for two hours in a control medium which lacked the substrate.

Following incubation, sections were fixed for 15 minutes in neutral buffered formalin, rinsed in distilled water and stained for three minutes in Grenacher's alum carmine. After staining, the sections were rinsed in distilled water and then the coverslips were mounted on glass slides with glycerol gelatine[6]. Sections treated in this manner show cells with carmine stained nuclei and fine, dark blue, diformazan granules (fig. 20). Some sections were not stained in alum carmine and, therefore, showed only diformazan granules.

The intensity of the diformazan deposition was assessed by visual microscopic examination of tissue sections and rated according to an arbitrary 0 to 6 scale where 0 represents no diformazan deposition and 6 the maximum reaction in a two hour incubation period. The intensity of the reaction was compared to photographs which represented the different divisions of the scale (fig. 21).

A few of the embryos were also examined for histochemically demonstrable reduced NAD:lipoamide oxidoreductase (diaphorase) using the method of Chayen and coworkers ('69). Frozen sections, serial to those used for 3β-HSD, were incubated at 38°C for two hours in the following medium:

Constituent	Volume	Weight
$NADH_2$[6] (3.0 mg/ml in distilled water)	0.8 ml	2.4 mg
Nitro-BT[7] (1.0 mg/ml in distilled water)	1.0 ml	1.0 mg
Phosphate Buffer, 0.1M, pH 7.2	5.2 ml	
Total Volume	7.0 ml	

[6] See footnote 5, page 34.
[7] See footnote 3, page 34.

Following incubation, sections were fixed for 15 minutes in neutral buffered formalin, stained with Grenacher's alum carmine, and then the coverslips were mounted on glass slides with glycerol gelatine. Sections treated in this manner show cells with carmine stained nuclei and fine, dark blue, diformazan granules. Some sections were not stained with alum carmine and these showed only diformazan granules.

II. Adrenal 3β-HSD activity in hypophysectomized chick embryos.

The eggs of embryos to be hypophysectomized and those of sham operated controls were opened after about 40 hours of incubation. A small hole was made through the shell and shell membranes on the underside of the egg. The egg was then turned so that the hole was uppermost and lying directly over the embryo. A hole was then made in the shell and outer shell membrane at the blunt end of the egg in order to permit air to escape from the air cell and to allow the formation of a new air chamber beneath the inner shell membrane at the uppermost side of the egg directly over the embryo. The embryo is thus lowered from the shell and shell membrane which can then be removed without injuring the embryo. By means of a small electric drill, fitted with an emery disc, a small square opening was made in the shell over the air chamber. The shell was then wiped with 70% alcohol to remove powdered shell. The shell membranes were then cut with a scalpel and the piece of shell removed. Embryos were hypophysectomized at stage 10 or 11 by the partial decapitation method of Fugo ('40). After cutting the vitelline membrane to expose the embryo, a traverse cut was made at the level of the mid-region of the mesencephalon (fig. 18) and the anterior portion of the embryo which contains the pituitary primordium completely removed (fig. 19). Embryos from

opened eggs with and without a cut through the prosencephalon or optic vesicles served as sham operated controls. Following the operation, the opening in the shell was ringed with melted paraffin and a warm glass coverslip placed on the paraffin to seal the opening thus forming a glass "window" in the egg-shell through which the embryo could be viewed. The hole in the blunt end of the egg was then sealed with paraffin and the egg returned to the incubator. The eggs were not turned during the remainder of the incubation period.

Hypophysectomized and sham operated control embryos were necropsied at stages 22 through 30 and at 24 hour intervals, from eight through 20 days of incubation, and examined for adrenal 3β-HSD activity using the procedures described in Experiment I.

III. Adrenal 3β-HSD activity in hypophysectomized embryos receiving adenohypophyseal chorioallantoic grafts.

Chick embryos were hypophysectomized by partial decapitation at stage 10 or 11 using the technique described in Experiment II. After 8.5 days of incubation the eggs were reopened and single, whole adenohypophyses, from 17 day old donors, were transplanted to the chorioallantoic membrane. The adenohypophyses were removed with a pair of sterile forceps and immediately placed on the host chorioallantoic membrane in the fork of a bifurcating vessel. The opening in the host egg was then resealed with melted paraffin and a glass coverslip and returned to the incubator. Controls consisted of hypophysectomized embryos which had received grafts of brain tissue. Donors of 17 days were employed since previous investigations had shown that the pituitary secretes ACTH during the final week of incubation (Case, '51, '52;

Adjovi, '70; and others).

The hypophysectomized embryos with chorioallantoic grafts were necropsied, at 24 hour intervals from nine through 19 days of incubation, and their adrenal glands recovered and examined for histochemically demonstrable 3β-HSD using the procedures described in Experiment I. The transplanted adenohypophyses were examined grossly to determine viability prior to removal of embryos from eggs. The transplants were then recovered, fixed and examined histologically in order to verify the presence of pituitary tissue.

IV. Adrenal 3β-HSD activity in hypophysectomized embryos receiving exogenous ACTH.

Chick embryos were hypophysectomized by partial decapitation at stage 10 or 11 using the technique described in Experiment II.

Lyophilized porcine ACTH[8], 30 IU/mg, was used in this experiment. Acidified saline (0.9% sodium chloride adjusted to pH 4.0 with 0.1N hydrochloric acid) served as the vehicle for the ACTH. After eight days of incubation, one International Unit (0.033 mg) of ACTH, dissolved in acidified saline, was injected through a needle-sized hole in the shell onto the chorioallantoic membrane adjacent to the embryo. Controls consisted of hypophysectomized embryos which had received an equal volume (0.05 cc) of the hormone vehicle. Injections were made at 12 and 24 hour intervals. The embryos were necropsied at 24 hour intervals, from nine through 19 days of incubation, and the adrenal glands recovered and examined for histochemically demonstrable 3β-HSD using the procedures described in Experiment I.

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[8] Supplied to Professor L. V. Domm through the courtesy of Dr. Russell L. Kutz Armour Pharmaceutical Company, Kankakee, Illinois.

RESULTS

More then 1700 white and brown Leghorn eggs were used in this investigation. These eggs yielded 1323 embryos of which number 194 were non-operated normal embryos, 720 were hypophysectomized, 349 were sham operated and 60 were used as adenohypophyseal donors (table 1).

A total of 528 embryos were only hypophysectomized and of this number 128 were alive at the time of necropsy. The survival rate was low owing to the drastic nature of the operation and approximately 24% of these embryos had survived at the time of necropsy. The mortality rate was highest during the first 36 hours after the operation. The survival rate for the treated hypophysectomized embryos, those receiving transplants or injections, was considerably higher due to the effects of treatment and the fact that survival rates were determined only from the time of treatment (about 8 days) and did not include the mortality immediately following hypophysectomy. The survival rate of non-treated hypophysectomized embryos was not determined after eight days of incubation.

Pregnenolone (P) and dehydroepiandrosterone (DHA) were used as substrates in determining 3β-hydroxysteroid dehydrogenase (3β-HSD) activity. The enzyme activity observed with the substrate pregnenolone was designated P-3β-HSD and that of dehydroepiandrosterone as DHA-3β-HSD. These activities were estimated by an evaluation of the density of diformazan deposition in the tissues (fig. 21).

I. Adrenal 3β-HSD activity in normal embryos and chicks.

A total of 170 normal embryos, ranging in age from 3.5 days of

incubation (stage 21) through hatching, and six chicks between one and 14 days of age were studied in this part of the investigation (table 2). One hundred and twenty-six embryos were quick frozen and examined histochemically for the presence of adrenal 3β-HSD activity and another 44 were fixed in Bouin's fluid, paraffin embedded, sectioned, and stained with hematoxylin and eosin to facilitate identification of non-stained frozen sections. All chick adrenals were examined for 3β-HSD activity. The DHA- and P-3β-HSD activity was determined by an evaluation of the diformazan (table 4). The average values for the embryonic period are shown in figure 1.

At stage 22 (3.5 - 4 days), the cells which eventually form the adrenal cortex have proliferated from the thickened peritoneal epithelium and are in the process of migrating dorsally between the dorsal aorta and the mesonephros. They can be seen in the mesenchyme immediately dorsal to the peritoneal epithelium (figs. 22, 23). Traces of DHA- and P-3β-HSD activity were observed in the migrating adrenocortical cells and in the thickened peritoneal epithelium (figs. 24, 25). These cells had no diformazan in control sections incubated in media lacking the substrate (fig. 26).

By stage 23 (4 days), some of the adrenocortical cells have completed their migration from the peritoneal epithelium and may be seen as scattered groups of two or three cells in the mesenchyme between the dorsal aorta and the mesonephros (fig. 27, 28). These cells had a P-3β-HSD activity of 2 and a DHA activity of 1 (figs. 1, 29, 30). These cells had no diformazan in control sections incubated in media lacking the substrate (fig. 31).

The DHA- and P-3β-HSD activity showed a steady increase from stage 23 (4 days) to stage 34 (8 days), however, the P-3β-HSD was always greater than

the DHA-3β-HSD activity (figs. 29-37). During this period both activities appeared to be evenly distributed throughout the cortical cell groups and cords.

From stage 30 through 34 (6.5 - 8 days) some of the adrenal glands showed indications of a zonation into peripheral and central cortical zones (figs. 38-42). In these adrenals the P-3β-HSD activity was higher in some of the peripheral than in the central cortical cords (figs. 38, 39). During this period there was an increase in the number of embryos showing adrenal zones of P-3β-HSD activity. Beginning at nine days and continuing through the remainder of the incubation period and up to 14 days after hatching, the last stage studied, all adrenal glands revealed a peripheral zone of high and a central zone of lower activity (figs. 48-50, 54-56, 60-62).

From 6.5 to 8 days of incubation there was also an increase in the number of peripheral cortical cords with a higher P-3β-HSD activity than the central cords and by nine days some of the adrenals had a peripheral zone of higher activity which extended completely around the gland. By ten days all adrenal glands had a complete peripheral zone of high P-3β-HSD activity.

At nine and ten days, the diameter of the central zone of some of the adrenals was about equal to the width of the peripheral zone (fig. 43). However, after ten days, there was a pronounced increase in the diameter of the central zone while the width of the peripheral zone remained relatively unchanged. During the last third of the incubation period and the first fourteen days after hatching, all adrenal glands showed a narrow peripheral

zone of high and a large central zone of lower P-3β-HSD activity (figs. 54, 60). In the peripheral zone, the activity was highest in the outer region adjacent to the capsule with a gradual decrease in activity from the outer peripheral to the central zone. The enzyme activity was evenly distributed throughout the cortical cords of the central zone.

When the zones first appeared at stage 30, the peripheral cortical cord P-3β-HSD activity was 6 and was about equal to that of the non-zonated adrenal glands of these stages (fig. 35) while the central zone activity was 5.0. The peripheral zone activity had decreased to an average of 5.3 at day eight (fig. 39) after which it gradually increased to a maximum of about 6.0 between 10 and 12 days (fig. 49) and again at the end of the incubation period (19 - 21 days) (fig. 56). Between 12 and 19 days the peripheral zone activity averaged about 5.0 (fig. 55). During the first two weeks after hatching this activity remained at the 6.0 level observed at the time of hatching.

The central zone P-3β-HSD activity reached a maximum average of 5.0 between 6.5 and 8 days (fig. 1) but after eight days of incubation there was a gradual decrease reaching its lowest level of 3.0 at the end of the incubation period (fig. 62). This level was maintained during the first fourteen days after hatching.

Some of the adrenal glands from stage 30 and 34 embryos showing a P-3β-HSD zonation also showed a zonation when examined for DHA-3β-HSD activity (figs. 41, 42). However, the distribution of this activity was just the opposite of that observed for P-3β-HSD, that is, the DHA-3β-HSD activity was greater in the central than in the peripheral zone (fig. 42). The later

was more difficult to detect but it was observed in most older embryos (figs. 46, 51, 57, 63) and in the post-hatch chicks. All of the adrenal glands showing a DHA-3β-HSD zonation also showed a P-3β-HSD zonation but not all of the latter showed a DHA-3β-HSD zonation.

In embryos of stages 30 to 34 (6.5 - 8 days) the central zone DHA-3β-HSD activity was essentially equal to the central zone P-3β-HSD activity (figs. 1, 39, 42). However, after eight days the central zone P-3β-HSD activity decreased to 3.0 while the DHA-3β-HSD increased and at 12 days it had reached a maximum of 6.0 which was maintained throughout the remainder of the incubation period (figs. 59, 65) and during the first two weeks after hatching. The peripheral zone DHA-3β-HSD activity increased steadily from an average of 4.0 at stage 30 to a level of 5.5 at the end of the incubation period (figs. 42, 47, 52, 58, 64). The 5.5 level observed at the time of hatching was also maintained in the post-hatch chicks. The peripheral zone DHA-3β-HSD activity was lower than the central zone P-3β-HSD activity from stage 30 (6.5 days) through 10 days and higher from 11 days through the remainder of the incubation period (table 4). The central zone DHA-3β-HSD activity was lower than the peripheral zone P-3β-HSD activity from 6.5 through 11 days and higher than the peripheral zone P-3β-HSD activity from 13 through 18 days (fig. 1). The DHA-3β-HSD activity in the non-zonated adrenal glands was equal to the central zone DHA-3β-HSD activity.

There was no evidence of the pink monoformazan in unstained sections from any of the embryos regardless of ages.

Most of the control sections incubated in media lacking the substrate had no nonspecific diformazan in the adrenal glands, however, some did have

trace amounts (figs. 26, 34, 37, 40, 45, 67). Nonspecific diformazan was found in control sections of the ependymal layer of the neural tube, intestinal epithelium, mesonephros, and liver.

Diformazan depositions of 1 and 2 intensity were observed in the adrenal chromaffin tissue of embryos of eight days and older. However, no diformazan was seen in the center of the large accumulations of chromaffin tissue found in older embryos (fig. 66). Control sections incubated in media lacking the substrate showed an absence or only trace amounts of diformazan in the chromaffin tissue.

No differences were observed in the distribution or degree of activity of adrenal DHA- or P-3β-HSD between brown and white Leghorn embryos.

Some embryos were also examined for histochemically demonstrable reduced NAD:lipoamide oxidoreductase (diaphorase). The diformazan deposition for diaphorase was considerably greater than that for DHA- or P-3β-HSD activity in all of the adrenal glands examined (figs. 68-73). In the adrenal glands of embryos through stage 32, the diaphorase activity was fairly evenly distributed throughout the cortical cords and the number of diformazan granules in each cell was so great as to almost completely obscure the nuclei (fig.68). In the adrenal glands of 10 and 20 day old embryos the diaphorase activity in the peripheral zone was so great that the diformazan formed an almost solid mass while the activity in the central zone was about equal to that seen in the younger embryos (figs. 68, 71, 74, 75). The 10 and 20 day embryos had typical DHA- and P-3β-HSD zonations (P-3β-HSD high in the peripheral zone, low in central zone: DHA-3β-HSD low in peripheral zone, high in central zone). The 11 day embryo also showed the typical adrenal 3β-HSD

zonation but showed no zonation when examined for diaphorase activity. The cortical cords in these adrenals were packed with diformazan.

II. Adrenal 3β-HSD activity in hypophysectomized embryos.

A total of 125 hypophysectomized and 100 sham operated control embryos, ranging in age from 3.5 (stage 21) through 20 days of incubation, were studied in this part of the investigation (table 2). One hundred and three of the hypophysectomized embryos and eighty three sham operated controls were examined for histochemically demonstrable adrenal 3β-HSD activity. The rest were fixed in Bouin's fluid, paraffin embedded, sectioned, and stained with hematoxylin and eosin for histological examination to facilitate identification of tissues in non-stained frozen sections.

No differences between normal, sham operated control or hypophysectomized embryos could be detected in the development of the adrenals from stage 22 (3.5 - 4 days) to nine days of incubation. Beginning at about ten and continuing to 19 days, the cortical cords of hypophysectomized embryos revealed hypertrophy and a reduction in number (figs. 76-79). During this period there was also an apparent increase in the amount of chromaffin tissue. Betz ('67) concluded that this increase in the chromaffin tissue of hypophysectomized embryos was only a relative increase since the adrenals of such embryos were smaller than normal and showed fewer cortical cords. The adrenal glands of hypophysectomized embryos from 10 through 19 days were more vascular than those of controls (fig. 76-79). The development of the adrenal glands of sham operated control embryos appeared identical to that of normal embryos.

The DHA- and the P-3β-HSD activity was determined by an evaluation of the diformazan deposition (tables 5, 6). The average values for hypophy-

sectomized embryos are shown in figure 2 and these compared with sham operated controls in figure 3.

Trace amounts of DHA- and P-3β-HSD activity were observed in the migrating adrenocortical cells and in the thickened peritoneal epithelium of hypophysectomized stage 22 (3.5 - 4 day) embryos. The scattered groups of adrenocortical cells in the mesenchyme between the dorsal aorta and the mesonephros of hypophysectomized stage 23 embryos had an average P-3β-HSD activity of 2.4 and an average DHA-3β-HSD activity of 1.1. These values were very close to the average of the control P- and DHA-3β-HSD values of 2.3 and 1.5, respectively (fig.3).

The P-3β-HSD activity of hypophysectomized embryos increased from 2.4 at stage 23 (4 days) to 5.5 at eight days of incubation while the DHA-3β-HSD activity increased from 1.1 to 4.5 during the same time period. These activities were about equal to those of sham operated control embryos (fig. 3). The P-3β-HSD activity was higher than the DHA-3β-HSD activity in all cases. These activities appeared to be evenly distributed throughout the cortical cell groups and cords during this period.

Beginning at eight days, in some of the adrenal glands of hypophysectomized and sham operated embryos, the P-3β-HSD activity was greater in some of the peripheral than in the central cords. Beginning at nine and continuing through 19 days, all the adrenal glands of sham operated embryos had a peripheral zone of high and a central zone of lower activity while during this period only about three fourths of the adrenals of hypophysectomized embryos showed indications of such a zonation (table 5, figs. 82-84, 92-94). At nine days, the number of peripheral cortical cords with a

high P-3β-HSD activity had increased to the point where they practically formed a complete peripheral zone in both hypophysectomized and sham operated control embryos. Control embryos of ten through 19 days had a complete peripheral zone which extended around the gland. In hypophysectomized embryos of this age, the cortical cords had hypertrophied, were reduced in number and separated from one another by large amounts of chromaffin tissue (figs. 74, 82). The peripheral cortical cords of these embryos did not form a complete zone of high activity because of the large amount of intervening chromaffin tissue. From ten through 19 days, the adrenal glands of hypophysectomized embryos showed a smaller central and a wider peripheral zone than those of corresponding ages. In many of the adrenal glands of these embryos, the width of the peripheral zone comprised the entire hypertrophied peripheral cortical cords.

The peripheral zone P-3β-HSD activity of hypophysectomized embryos was about equal to that of controls from eight through 11 days and slightly lower from 12 through 19 days except for a slight increase at days 17 and 18 (fig. 3a). There was a decrease in the central zone P-3β-HSD activity of these embryos from 5.2 at 8 days to 4.5 at 13 days after which there was a leveling off at about the 4.5 level (fig. 3a). The central zone P-3β-HSD activity of control embryos decreased from 4.9 at 8 days to 3.4 at 19 days of incubation.

About one fourth of the adrenal glands of hypophysectomized embryos from eight through 19 days showed no indication of a zonation when examined for P-3β-HSD activity (table 5, figs. 80, 89). At eight and nine days this activity was evenly distributed throughout the cortical cords. From ten

through 19 days, these cords showed hypertrophy and an even distribution of P-3β-HSD activity whose intensity approximated that of the peripheral zone of hypophysectomized embryos (fig. 3a).

Beginning at eight days of incubation the DHA-3β-HSD activity was greater in the central than in some of the peripheral cords of some of the adrenal glands of hypophysectomized and sham operated control embryos (table 6). From nine through 19 days, about two thirds of the adrenal glands of hypophysectomized embryos showed peripheral cords with a lower activity than that of the central cords (figs. 85-87, 95-97). Most, but not all of these adrenals also showed a P-3β-HSD zonation. From ten through 19 days, the peripheral zone was not continuous owing to the large amount of intervening chromaffin tissue separating the hypertrophied peripheral cortical cords (figs. 85, 95). The peripheral zone DHA-3β-HSD activity in the adrenal glands of hypophysectomized embryos was about equal to that of sham operated control embryos whereas the central zone DHA-3β-HSD activity of such embryos was about equal to that of controls from eight through 12 days but about 1.0 lower than that of controls throughout the remainder of the incubation period (table 6, fig. 3b). In the adrenal glands of hypophysectomized embryos the central zone DHA-3β-HSD activity was less than the peripheral zone P-3β-HSD activity between eight and 11 days and between 16 and 19 days and equal to the P-3β-HSD activity between 12 and 15 days (table 6, fig. 2). In the adrenal glands of sham operated control embryos the central zone DHA-3β-HSD activity was about 1.0 less than the peripheral zone P-3β-HSD activity up to 12 days and between 13 and 19 days it was equal to the P-3β-HSD activity (table 6, fig. 4).

Between 15 and 20 days of incubation, a few hypophysectomized embryos

had adrenal glands with a peripheral zone of high and a central zone of lower DHA-3β-HSD activity. In these adrenal glands the P-3β-HSD activity was also higher in the peripheral than in the central zone. In normal and sham operated control embryos, DHA-3β-HSD activity was always higher in the central zone than in the peripheral zone.

About one fourth of the adrenal glands of hypophysectomized embryos, when examined for DHA-3β-HSD activity, showed no indication of a zonation between eight and 19 days of incubation (table 6, figs. 80, 89). At eight and nine days this activity was evenly distributed throughout the cortical cords in the central and peripheral areas of the gland. From ten through 19 days, the cortical cords revealed hypertrophy and the DHA-3β-HSD activity of these non-zonated adrenals was somewhat lower than that of the central zone DHA-3β-HSD activity of hypophysectomized embryos except after 17 days, when it was higher (fig. 2).

The DHA- and P-3β-HSD activity in the adrenal glands of normal and sham operated control embryos is shown in figure 5. This activity in sham operated control embryos increased steadily from stage 23 (4 days) to eight days and was approximately equal in intensity to that of normal embryos. The P-3β-HSD activity was always greater than the DHA-3β-HSD activity at these ages. Beginning at eight days of incubation the adrenal glands of control embryos showed the first indications of a zonation. This was approximately 24 hours earlier (stage 30, 6.5 - 7 days) in the adrenal glands of normal embryos. By ten days, all the adrenal glands of normal and sham operated embryos showed a complete peripheral zone of high P-3β-HSD activity and a central zone of lower activity. This peripheral zone activity, in the adre-

nal glands of normal and control embryos, reached a maximum of about 6 between ten and 12 days then showed a gradual decrease to a low of 5.0 at 18 days after which there was an increase to the maximum level of 6 (fig. 5a). The central zone P-3β-HSD activity of these embryos decreased steadily from eight days through the remainder of the incubation period. The central zone P-3β-HSD activity of sham operated control embryos decreased from an average of 5.2 at eight days to an average of 3.4 at 19 days while that of normal embryos decreased from 5.0 to 3.1 during this period (fig. 5).

The peripheral zone DHA-3β-HSD activity in the adrenal glands of sham operated embryos revealed the same intensity and distribution as that of normal embryos from eight through 19 days (fig. 5b). The central zone DHA-3β-HSD activity of sham operated embryos was approximately the same as that of normal embryos from eight through 14 days and then somewhat less than normal up to 19 days.

A small percentage of the adrenal glands of both normal and sham operated control embryos showed no indication of a zonation during the later stages of incubation.

III. Adrenal 3β-HSD activity in hypophysectomized embryos with adenohypophyseal chorioallantoic grafts.

A total of 34 hypophysectomized embryos which had received adenohypophyseal chorioallantoic grafts (H+A embryos) and 11 which had received a small piece of brain (H+B embryos), ranging in age from nine through 19 days of incubation, were studied in this part of the investigation (table 3). Twenty eight of the H+A and all of the H+B embryos were examined for histochemically

demonstrable 3β-HSD activity. The remaining H+A embryos were fixed in Bouin's fluid, paraffin embedded, sectioned, and stained with hematoxylin and eosin for histological examination to facilitate identification of unstained frozen sections. The grafts which were recovered were prepared in the same manner to determine presence of viable graft tissue.

There was considerable variation in the histological appearance of the adrenal glands of H+A embryos which appeared to be related to the amount and condition of the grafted adenohypophyseal tissue. The histological appearance of adrenal glands from embryos with grafts containing well developed adenohypophyseal tissue resembled those of normal and sham operated embryos. The majority of these embryos had grafts which contained well developed adenohypophyseal tissue (figs. 99-101). A few had grafts which contained a very small amount of adenohypophyseal tissue in some of which this tissue was undergoing degeneration (figs. 102-103). The adrenal glands of these embryos revealed many of the characteristics of those of hypophysectomized embryos i.e. various degrees of hypertrophy of the cortical cords, a reduction in their number, increased amounts of chromaffin tissue and an increase in vascularity. The histological appearance of the adrenal glands of H+B embryos was identical to those of hypophysectomized embryos.

The DHA- and P-3β-HSD activity in the adrenal glands of H+A and H+B embryos was, as above, determined by an evaluation of diformazan deposition (tables 7, 8). The average values are shown in figure 6. The average values of the 3β-HSD activity of H+A embryos were compared with those of H+B embryos (figs. 7a, 8a) and sham operated embryos (figs. 7b, 8b). The average values

of H+B embryos were also compared with those of non-treated hypophysectomized embryos (fig. 9).

The distribution of both DHA- and P-3β-HSD activity in the adrenal glands of H+A embryos resembled the distribution in normal and sham operated embryos (figs. 104, 107, 110, 113). The size of the peripheral and central zones were normal.

In all of the H+A embryos, the adrenal P-3β-HSD activity was higher in the peripheral than in the central cortical cords (table 7, figs. 104-106, 110-112). The peripheral zone activity increased from an average of 5.5 at nine days to a maximum of 6 from 11 through 13 days then gradually decreased to 5.0 at 18 days and had again increased to 5.7 at 19 days (fig. 6a). The central zone P-3β-HSD activity showed a steady decrease from 5.0 at nine to 3.2 at 19 days.

Some of the adrenal glands of H+B embryos were zonated and some non-zonated. The P-3β-HSD activity of the non-zonated ones was higher than that of the peripheral zone of the zonated ones (table 7, fig. 6b). The average P-3β-HSD activity of the zonated adrenal glands was very close to that of the non-treated hypophysectomized embryos (fig. 9a).

The peripheral and central zone P-3β-HSD activity levels of H+A embryos were maintained at levels characteristic of sham operated embryos (fig. 7b) rather than at levels characteristic of non-treated hypophysectomized embryos (fig. 7a).

In a majority of the adrenal glands of H+A and H+B embryos, the DHA-3β-HSD activity was higher in the central zone than in the peripheral zone

(table 8). The central zone activity of H+A embryos increased from an average of 5.0 at nine days to a maximum of 6 at 15 days then showed a decrease to 5.5 at 18 days (fig. 6a) while that of the peripheral zone showed a steady increase from 4.2 at nine days to 5.5 at 15 days then a decrease to 4.0 at 18 days.

In H+B embryos the average central zone DHA-3β-HSD activity was about equal to that of non-treated hypophysectomized embryos while the peripheral zone activity averaged about 0.4 less than that of non-treated hypophysectomized embryos (fig. 9b).

The peripheral zone DHA-3β-HSD activity of H+A embryos was about equal to that of non-treated hypophysectomized and sham operated embryos (fig. 8) while the central zone activity of H+A embryos was maintained at levels characteristic of sham operated embryos (fig. 8b) rather than at levels characteristic of non-treated hypophysectomized embryos (fig. 8a).

The adrenal glands of H+A embryos which did not appear to be zonated had an overall DHA-3β-HSD activity equal to the central zone of the zonated ones (fig. 6a).

The adrenal glands of 19 day H+A embryos showed a peripheral zone of high (5.7) and a central zone of lower (5.0) DHA-3β-HSD activity. In the adrenals of these embryos, the average peripheral zone DHA- and P-3β-HSD activities were both 5.7 but the central zone P-3β-HSD activity$_{\wedge}^{(3.2)}$ was considerably less than the DHA-3β-HSD activity (5.0) (tables 7, 8, fig. 6a).

IV. Adrenal 3β-HSD activity in hypophysectomized embryos injected with ACTH.

A total of 77 hypophysectomized embryos received ACTH at either 12 (H+ACTHx2) or 24 hour intervals (H+ACTHx1 embryos). Of the 63 ACTH treated embryos which were alive at the time of necropsy, 59 were examined for histochemically demonstrable 3β-HSD activity and 4 were processed for histological examination to facilitate identification of unstained tissues in frozen sections (table 1). Twenty-five hypophysectomized embryos received injections of hormone vehicle (acidified saline) at the same periods and volume and served as controls. No differences were observed between the adrenal glands of embryos which received the vehicle at 12 hour and those which received it at 24 hour intervals. The embryos which received only the vehicle are designated as H+VEH embryos. Twenty of the latter were alive at the time of necropsy and were processed for 3β-HSD activity. The H+ACTH and H+VEH embryos were necropsied at 24 hour intervals from nine through 19 days of incubation (table 3).

There was considerable variation in the appearance of the adrenal glands of embryos which received ACTH. In hypophysectomized embryos which had received 1.0 IU of ACTH at 12 hour intervals (2.0 IU per day), the overall histological appearance of the adrenal glands was that of normal embryos, however, the peripheral cortical cords showed some hypertrophy and the glands were somewhat more vascular in a few of the embryos. None of these adrenal glands resembled the typical adrenal glands of hypophysectomized embryos. On the other hand, all of the adrenal glands of embryos which had received the hormone vehicle (H+VEH embryos) were similar to those of non-treated

hypophysectomized embryos, i.e., the cortical cords were hypertrophied and reduced in number, showed a greater accumulation of chromaffin tissue and varying increases in vascularity.

The histological appearance of the adrenal glands of embryos which had received 1.0 IU of ACTH at 24 hour intervals (H+ACTHx1 embryos) was considerably more variable than those of H+ACTHx2 embryos. Up through 12 days, the adrenal glands of H+ACTHx1 embryos appeared normal, however, the cortical cords of a few showed some hypertrophy. After 12 days a few of the adrenal glands were normal, a few were similar to those of hypophysectomized embryos and the appearance of the rest was somewhere between those of normal and those of hypophysectomized embryos.

The DHA- and P-3β-HSD activity, as in the other groups, was determined by an evaluation of the diformazan deposition (tables 9-11). The average values of adrenal 3β-HSD activity of H+ACTH embryos are shown in figure 10 and are compared with those of H+VEH embryos (figs. 11, 14), with non-treated hypophysectomized embryos (figs. 12, 15), and with sham operated controls (figs. 3, 6). In addition, the average 3β-HSD activity of H+VEH embryos was compared with those of non-treated hypophysectomized embryos (fig. 17).

The distribution of both DHA- and P-3β-HSD activity in the adrenal glands of H+ACTHx2 embryos resembled the distribution in normal and sham operated embryos (figs. 116, 119, 122, 125).

In all of the H+ACTH embryos of 10 through 19 days incubation, the adrenal P-3β-HSD activity was higher in the peripheral than in the central cortical cords (tables 9, 10, figs. 117, 118, 123, 124). In both H+ACTHx1

and H+ACTHx2 embryos the peripheral zone activity was at the maximum level of 6.0 at 10 days, had decreased to around 5.0 at 16 and 17 days, then increased to 5.5 towards the end of the incubation period (fig. 10a).

The peripheral zone P-3β-HSD activity of H+VEH embryos was at a maximum level of 6.0 at nine and 11 days of incubation (fig. 17a). From 12 to 16 days, the last stage of H+VEH embryos examined, this activity decreased steadily to 5.0 except for an increase to 6.0 at 14 days. The peripheral zone activity of H+VEH embryos equaled that of non-treated hypophysectomized embryos at 10, 11, 15 and 16 days (fig. 17a). From 12 to 14 days, the activity of H+VEH embryos paralleled that of hypophysectomized embryos but was about 0.5 higher. The peripheral zone P-3β-HSD activity levels of H+ACTHx1 embryos between 10 and 16 days and H+ACTHx2 embryos between 10 and 18 days were maintained at levels characteristic of sham operated embryos (fig. 13) rather than at the levels characteristic of non-treated hypophysectomized (fig. 12) or H+VEH embryos (fig. 11). The peripheral zone of high activity was continuous in most of the H+ACTHx2 embryos, however, some of these embryos did have hypertrophied peripheral cortical cords with intervening chromaffin tissue so that they did not show a continuous peripheral zone of high activity. A majority of the H+ACTHx1 embryos had adrenal glands in which the peripheral zone of high activity also was not continuous.

The average central zone P-3β-HSD activity of H+ACTHx1 embryos was 5.0 at 10 and 11 days of incubation, decreased to about 4.4 from 12 to 14 days, and then increased to about 5.0 from 15 through 18 days (fig. 10a). The central zone activity of H+ACTHx2 embryos was at the maximum level of about 5 on days nine and ten, then showed a decrease to about 4.2 from day 11

through 17 and a further decrease to 3.5 on day 18 (fig.10a). From 11 through 18 days of incubation, the central zone activity of the H+ACTHx2 embryos was lower than that of the H+ACTHx1 embryos except on day 12 when it was about the same.

The central zone P-3β-HSD activity of H+VEH embryos was at a maximum level of 5.5 on days nine and 11 after which there was a gradual decrease to 4.5 on days 15 and 16. These levels were somewhat higher than those of nontreated hypophysectomized embryos between nine and 14 days and the same on days 15 and 16.

The central zone P-3β-HSD activity of the H+ACTHx1 embryos from 11 through 14 days was lower than that of the H+VEH embryos (fig. 11a) and about equal to that of the sham operated embryos (fig. 13a). After 14 days the central zone P-3β-HSD activity of H+ACTHx1 embryos was higher than that of sham operated embryos.

The central zone P-3β-HSD activity levels of the H+ACTHx2 embryos were at levels characteristic of the sham operated embryos (fig. 13b) rather than those of non-treated hypophysectomized (fig. 12b) or H+VEH embryos (fig. 11b).

About 80% of the adrenal glands of H+ACTHx1 embryos had a central zone of high and a peripheral zone of lower DHA-3β-HSD activity while only about 40% of the adrenals of the H+ACTHx2 embryos showed such a zonation.

The average central zone DHA-3β-HSD activity of H+ACTHx2 embryos was about 0.5 higher than that of H+ACTHx1 embryos from ten through 14 days and about the same after 15 days (fig. 10b). There was very little difference between the average central zone DHA-3β-HSD activities of the H+ACTH, H+VEH,

and the non-treated hypophysectomized embryos up through between 15 and 16 days of incubation (figs. 14, 15, 17). After 16 days of incubation the central zone DHA-3β-HSD activity of the H+ACTHx1 and H+ACTHx2 embryos was somewhat higher than that of non-treated hypophysectomized embryos (fig. 15). The central zone DHA-3β-HSD activity of both groups of H+ACTH embryos appeared to be less than that of sham operated embryos between 12 and 16 days of incubation but before and after this period the activity was about the same (fig. 16).

The average peripheral zone DHA-3β-HSD activity of H+ACTHx2 embryos was about 0.5 higher than that of the H+ACTHx1 embryos from ten through 14 days and was about the same after 15 days (fig. 10b). On days ten and 11 the peripheral zone activity of the H+ACTHx1 embryos was 0.5 higher than that of the H+VEH embryos (fig. 14a) but was the same as that of non-treated hypophysectomized embryos (fig. 15a). Peripheral zone DHA-3β-HSD activity of the H+ACTHx2 embryos averaged about 0.5 higher than that of the H+VEH from nine through 14 days and about the same after 15 days (fig. 14b). Average central zone DHA-3β-HSD activity of H+ACTHx1 embryos was about the same as that of sham operated embryos except on days 13 through 15 when the average of the H+ACTHx1 embryos was about 5.0 and that of the sham operated embryos from 5.7 to 6 (fig. 16a). The average central zone DHA-3β-HSD activity of H+ACTHx2 embryos was about the same as that of sham operated embryos (fig.16b)

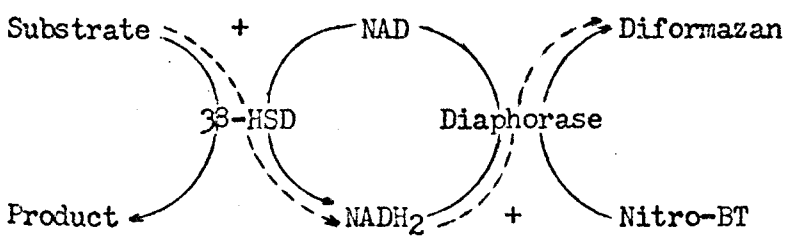
In four adrenal glands of H+ACTH embryos the DHA-3β-HSD activity was higher in the peripheral than in the central zone. In these embryos the peripheral zone DHA-3-HSD activity was about equal to the peripheral zone

P-3β-HSD activity but the central zone DHA-3β-HSD activity was equal to the central zone DHA-3β-HSD activity of normally zonated adrenals.

About 20% of the adrenal glands of H+ACTHx1 embryos and 60% of the H+ACTHx2 embryos did not appear to be zonated when examined for DHA-3β-HSD activity (tables 9, 10). In many of these the diformazan deposition was very evenly distributed throughout the gland and the minor variations in the density of deposition which occurred in normal adrenal glands were lacking.

DISCUSSION

The histochemical demonstration of steroidogenic enzymes enables one to identify cells with a steroidogenic potential. Wattenberg ('58) developed a histochemical method which enables one to determine the enzyme reaction in which pregnenolone is converted to progesterone or dehydroepiandrosterone (DHA) to Δ^4-androstenedione. Two enzymes, 3β-hydroxysteroid:NAD oxidoreductase (3β-hydroxysteroid dehydrogenase or 3β-HSD) and 3-ketosteroid Δ^4- Δ^5-isomerase, are involved in this transformation. 3β-HSD oxidizes the 3β-hydroxyl group requiring nicotinamide adenine dinucleotide (NAD), and 3-ketosteroid Δ^4- Δ^5-isomerase catalyzes the migration of the double bond from the 5-6 position to the 4-5 position. Wattenberg ('58) incubated unfixed frozen sections in a medium containing the substrate (dehydroepiandrosterone or pregnenolone) dissolved in acetone, plus NAD, nicotinamide, a tetrazolium salt (Nitro-BT) and buffer (pH 8.0). Hydrogen ions removed from the substrate by 3β-HSD are transferred to NAD forming $NADH_2$, then from $NADH_2$ to the tetrazolium salt. The tetrazolium salt will not accept the hydrogen directly and its reduction is attributed to reduced-NAD:lipoamide oxidoreductase (diaphorase) which is present in the tissue. The scheme of hydrogen transport in this reaction is as follows:



The broken line shows the transport of hydrogen.

The tetrazolium salt is colorless and water soluble in its unreduced form, but when reduced it becomes colored and insoluble, and is deposited in the form of fine diformazan granules at the site of the enzyme reaction.

In all of the chick adrenal glands examined for diaphorase, the diformazan deposition, due to the activity of diaphorase, was many times greater than the deposition due to the 3β-HSD-diaphorase system. Since the substrate, NAD and Nitro-BT were present in the media in excessive amounts and large amounts of endogenous diaphorase were also present, the limiting factor in the above reaction for demonstrating the presence of 3β-HSD is the amount of the enzyme present in the tissue.

Levy, Deane and Rubin ('59) modified Wattenberg's method by using proplyene glycol as the substrate solvent, lowering the pH of the buffer and changing the concentration of NAD, Nitro-BT and the substrate.

The media used in this investigation was a slight modification of the method of Levy, Deane and Rubin ('59). Nicotinamide, which was used by these investigators to protect the NAD from enzymatic destruction, was not present in the media used in our investigation. Baillie, Ferguson and Hart ('66) maintain that the nicotinamide in the medium is of doubtful value. Nicotinamide was not present in the media used by Woods and Domm ('66).

Diformazan granules, indicating the presence of 3β-HSD activity, were observed in the adrenocortical cells of stage 22 (3.5 - 4 day) embryos. At this stage, the cells which eventually form the adrenal cortex have proliferated from the thickened peritoneal epithelium and are seen in the mesenchyme immediately dorsal to the epithelium and migrating dorsally between

the dorsal aorta and the mesonephros. Straznicky, Hajos and Bohus ('66) assayed chick adrenal homogenates for 3β-HSD activity and first detected this enzyme in 14-15 day embryos. Using histochemical methods, Sivaram ('64) first observed the enzyme in ten day embryos, Boucek, Györi and Alvarez ('66) at five days, and Chieffi, Manelli, Botte and Mastrolia ('64) at 4.5 days. These discrepancies in observations on the time of appearance of this enzyme in the embryonic adrenal may, in part, be attributable to variations in the early development of chick embryos. Cognizant of these variations, Dawson ('53) suggested that comparisons, of observations on the chick from different laboratories, be made with caution owing to the variables inherent in a system of chronological rating, despite a rigorous standardization of local conditions. Since we observed considerable variation at early ages, in the development of embryos of comparable incubation ages, we employed morphological characteristics (Hamburger and Hamilton, '51) in determining the age of embryos prior to eight days of incubation.

The discrepancies in observations on the time of appearance of 3β-HSD in the adrenal gland of the chick may also be attributable to variations in the histochemical techniques employed in demonstrating this emzyme. Boucek, Györi and Alvarez ('66) found the first evidence of adrenal 3β-HSD activity in 6μ sections of five day embryos using pregnenolone dissolved in acetone-buffer as the substrate. The incubation time was not reported; however, just after the incubation a lipid extraction was made which "removed most of the fat and the crystals of formazan precipitates, leaving discrete punctate deposits." In our investigation, trace amounts of adrenal 3β-HSD

were observed in 10μ sections of stage 22 (3.5 - 4 days) embryos using pregnenolone and DHA dissolved in propylene glycol as the substrate and incubating for two hours without lipid extraction. At stage 26 (5 days) P-3β-HSD was present in all adrenal glands and the activity averaged about 4.0. The presence of acetone in the media, the thickness of the tissue sections, the length of incubation and the lipid extraction may be key factors involved in the detection of minimal amounts of the enzyme.

Since the adrenocortical cells arose from the thickened peritoneal epithelium which revealed 3β-HSD activity at stage 21 (Domm and Ericson, '72), it was not surprising to find this enzyme in the migrating adrenocortical cells. 3β-HSD activity was detected in the mouse adrenocortical anlagen as soon as it became recognizable in the 12 day embryo as a group of cells immediately above the peritoneum (Hart et al., '66).

In this investigation the P-3β-HSD activity of the adrenocortical cells of stage 22 through 30 (3.5 to 7 days) embryos was higher than the DHA-3β-HSD activity. From stage 32 (7.5 days) through hatching, the central zone DHA-3β-HSD activity was higher than the central zone P-3β-HSD. The only other investigation we know of in which both pregnenolone and dehydroepiandrosterone (DHA) were used as substrates, in studying 3β-HSD activity in the embryonic chick adrenal gland was that of Boucek, Györi and Alvarez ('66). These investigators, however, only reported that these cells displayed a prominent reaction with pregnenolone and a less intense reaction with DHA at 9 and 11 days.

Although the cortical and medullary tissues are intermingled in the

avian adrenal gland, the results of this investigation indicate that the cortical cords of the chick embryo are divided into a narrow peripheral zone and a large central zone. Other investigators have also reported that the cortical tissue at the periphery of the avian adrenal differs from that more centrally located. Miller and Riddle ('42) reported a gross difference in the cortical cell structure between periphery and center of the pigeon adrenal gland. Five years later, Kar ('47) made similar observations in the chicken reporting that the cortical cells at the periphery were large, had a considerable amount of cytoplasm and nuclei which were rarely pyknotic. The cortical cells of the central zone were smaller with somewhat smaller nuclei many of which were pyknotic. Arvy ('61) in work on the adrenal glands of the domestic fowl, observed that there were higher concentrations of histochemically demonstrable neutral fats in the cortical tissue of the central than in the peripheral zone.

Kondics and Kjaerheim ('66) reported that the morphology of the mitochondria in the thin subcapsular zone of cortical tissue in the chicken adrenal gland were different from the mitochrondria in the broad inner zone. Similarities in morphology and distribution of the mitochondria in the adrenal glands of mammals (rat, mouse, and cow) with those of the adrenal glands of the bird, suggest that the subcapsular (peripheral) zone and the inner (central) zone of the avian adrenal gland are homologous with the zona glomerulosa and zona fasciculata, respectively, of the mammalian adrenal gland (Frankel, '70).

It has been reported that in the chick embryo beginning at about 15 days of incubation, the cortical cords of the peripheral zone show a greater

abundance of mitochondria and higher concentrations of ascorbic acid, alkaline phosphomonesterase, aliesterase and cholinesterase than those of the central zone (Sivaram, '64, '65, '68). The results of our investigation indicate that zonation of the embryonic chick adrenal gland begins at about 7 to 7.5 days of incubation (stage 30 to 32) and was seen in all adrenal glands by nine days. At this time the cortical cords of the peripheral zone had a high P-3β-HSD and a low DHA-3β-HSD activity while those of the central zone had a low P-3β-HSD and a high DHA-3β-HSD activity. There is a close correlation between the initial appearance of the adrenocortical zones at eight days, the onset of adrenocorticotropic activity of pituitary extracts at eight days (Székely, Endroczi and Szentagothai, '58) and the initiation of corticoid secretion in the adrenal glands of eight day old chick embryos (Pedernera, '71). Toth, Simon and Székely ('58) have observed that there is a significant increase in the number of mitoses in the adrenal cortex of normal chick embryos on the seventh day of incubation (stages 29 and 30). Since this increase did not occur at this time in hypophysectomized embryos, these authors concluded that the increase in mitoses was a consequence of the onset of ACTH secretion by the pituitary.

We observed an increase in P-3β-HSD activity from stage 22 (3.5 - 4 days) through stage 32 (7.5 days) the time at which zonation appears. After eight days of incubation the peripheral zone activity remained high while that of the central zone gradually decreased up to the the time of hatching (20 - 21 days). During the first 14 post hatch days the P-3β-HSD activity remained at the hatching time level. Boucek, Győri and Alvarez ('66) observed that adrenal P-3β-HSD activity, which was present after

five days of incubation, attained a maximum between 11 and 12 days. The activity decreased on day 13 and remained at this level to the time of hatching and through the first 14 days after hatching. These investigators did not mention the presence of zones. Chieffi, Manelli, Botte and Mastrolia ('64) observed DHA-3β-HSD activity in chick adrenal glands from 4.5 days of incubation to four days after hatching but they also did not mention the presence of cortical zones. In adult chickens, histochemically demonstrable DHA-3β-HSD activity was found to be higher in the central than in the peripheral cortical zone (Arvy, '62).

The differences in the intensities and distribution of DHA- and P-3β-HSD activities in the adrenal gland of the chick suggest the presence of two substrate-specific 3β-hydroxysteroid dehydrogenases. The existence of different substrate-specific 3β-hydroxysteroid dehydrogenases has also been suggested by Baillie and Griffiths ('64) as a result of observations on the Leydig cells of the fetal mouse. Their results suggest that the 3β-HSD concerned with the conversion of pregnenolone to progesterone in these cells was first to develop at about 11 days followed five days later by the enzyme concerned with the metabolism of DHA to Δ^4-androstenedione. The investigation of Boucek, Györi and Alvarez ('66) on the gonadal tissues of the chick embryo also suggests the presence of two substrate specific 3β-hydroxysteroid dehydrogenases. These investigators observed that in sections of ovarian tissue, the medullary sex cords had an intense reaction with pregnenolone and a somewhat less intense reaction with dehydroepiandrosterone while the cells of the primary albuginea and stroma had an intense reaction with dehydroepiandrosterone but no reaction with pregnenolone.

We observed granules of diformazan, indicating the presence of 3β-HSD activity, in what appears to be the adrenal chromaffin tissue (medulla) of eight day and older embryos. No diformazan was observed in the center of large accumulations of chromaffin tissue of older normal or hypophysectomized embryos. The chromaffin tissue (medulla) of mammals is completely negative for 3β-HSD activity (Wattenberg, '58) and Chieffi, Manelli, Botte and Mastrolia ('64) reported that 3β-HSD activity (DHA substrate) in the chick embryo appeared only in the cortical cords. The positive reaction seen in the present investigation could be a diffusion artifact or superimposition of the activity in the cortical cells over the small groups of chromaffin cells. Wattenberg ('58) has shown that diffusion of the enzyme, $NADH_2$ or diformazan does not occur. Therefore, we feel that the activity appearing in the chromaffin tissue is, in fact, in the cortical cords overlying the small groups of chromaffin cells. In those areas where there were large accumulations of chromaffin tissue, the entire thickness of the section consisted of chromaffin cells, hence, no diformazan was present.

Hypophysectomy of chick embryos by the partial decapitation method of Fugo ('40), at about 40 hours of incubation, removed both the pituitary and hypothalamic primordia. Normal development of the adrenal glands of the chick embryo does not appear to be dependent on the embryonic pituitary gland prior to ten days of incubation. Beginning at ten and continuing through 19 days, the adrenals of hypophysectomized embryos revealed fewer and larger than normal cortical cords, larger than normal accumulations of chromaffin cells and an increase in vascularity. These observations on the appearance of the

adrenal glands of hypophysectomized chick embryos are in agreement with those of Betz (67) on 20 day old hypophysectomized chick embryos.

Hypophysectomy also had no apparent effect on either the P- or the DHA-3β-HSD activity in the adrenal glands prior to nine days of incubation. The first indications of a zonation were observed at eight days in both hypophysectomized and sham operated control embryos. However, from nine through 19 days, all the adrenal glands of sham operated embryos revealed P-3β-HSD zones and about 91% also had DHA-3β-HSD zones while in hypophysectomized embryos only about 75% of the adrenal glands showed P-3β-HSD zones and 66%, DHA-3β-HSD zones. In the hypophysectomized embryos the peripheral zone appeared wider and the central zone smaller than those of sham operated embryos. In the zonated adrenal gland of hypophysectomized embryos, the P-3β-HSD activity was somewhat lower in the peripheral zone and higher in the central zone than the corresponding zones in the adrenal glands of sham operated embryos. Peripheral zone DHA-3β-HSD activity in the adrenal glands of hypophysectomized embryos was about equal to that of sham operated while the central zone activity was somewhat less. Some of the adrenal glands of hypophysectomized embryos revealed no evidence of zones. The P-3β-HSD activity of these non-zonated adrenals was about equal to the peripheral zone activity of hypophysectomized embryos. These observations are not in agreement with those of Manelli ('64) who observed no effect of hypophysectomy on histochemically demonstrable adrenal 3β-HSD activity (substrate not reported) in chick embryos after 12 to 15 days of incubation. Straznicky, Hajos and Bohus ('66), on the other hand, observed biochemically detectable 3β-HSD activity in 14 to 15 day old chick embryos, but observed no activity in 17 day old embryos which had been

hypophysectomized by decapitation on the second day of incubation.

Samuels and Helmreich ('56) found that biochemically detectable 3β-HSD activity in homogenated adrenal glands of hypophysectomized rats decreased with atrophy of the glands but the activity per unit mass did not drop significantly. Levy, Deane and Rubin ('59) observed that the involuting inner cortex in the adrenals of hypophysectomized adult rats revealed normal levels of histochemically demonstrable 3β-HSD three days after surgery but thereafter there was a gradual decrease in activity and by 50 days almost all activity had disappeared. The retention of lactic dehydrogenase in many cells of the inner cortex at this time indicated that they had not yet become moribund. After 50 days the 3β-HSD activity in the outer cortical zone appeared to be normal or somewhat enhanced.

Miller ('67) measured the effects of adenohypophysectomy at 1.9 to 2.4 months, upon the weight of chromaffin tissue, and of central and peripheral interrenal (cortical) tissue in pigeons adenohypophysectomized for as long as 128 days. Removal of the pars distalis evoked a slight decrease in total adrenal weight. The weight of the cortical tissue decreased progressively while that of the chromaffin tissue showed a slight increase. Atrophy of the cortical tissue was confined to the central part of the gland while the weight of the peripherally located cortical tissue remained unchanged for up to 128 days. Frankel, Graber and Nalbandov ('67α) made similar observations on the adrenal glands of adenohypophysectomized cockerels and also reported that the peripheral cortical tissue appeared to be stimulated. Manelli and Mastrolia ('63) observed that after culturing the adrenal glands of 12 day

old chick embryos for ten days, in media which lacked ACTH, some DHA-3β-HSD activity was retained in the peripheral cortical tissue of some of the adrenal glands while no activity was observed in the central cortical tissue.

The modifications in the avian adrenal glands following adenohypophysectomy resemble those seen in the adrenals of hypophysectomized rats. In the rat, hypophysectomy leads to atrophy of the two inner zones (zonae fasciculata and reticularis) of the adrenal cortex. There is a reduction in both the size and the number of cells and after two months, the zona fasciculata cannot be distinguished from the zona reticularis (Greep and Deane, '49). The zona fasciculata appears to be completely dependent on the activity of the pituitary for the maintenance of cell size and for the formation of its secretions (Greep and Deane, '49). Unlike the inner zones of the cortex, the outer zona glomerulosa appears to be largely independent of pituitary control. Following hypophysectomy, the cells of the zona glomerulosa maintain a healthy appearance and their volume appears to undergo little change, however, the width of the zona glomerulosa broadens as the inner zones atrophy (Deane and Greep, '46; Deane, '62).

Additional support for the concept of the homology of the zones in the avian and mammalian adrenal glands, has been based upon the similarities in response to experimental conditions other than adenohypophysectomy. Taylor, Davis, Breitenbach and Hartroft ('70) observed that chronic sodium depletion in the chicken was associated with hypertrophy and hyperplasia of the peripheral cortical zone of the adrenal gland. A similar response was observed in the zona glomerulosa of the adrenal glands of sodium deficient rats (Deane, Shaw,

and Greep, '48). However, there is an increase in the production of aldosterone by the adrenals of sodium deficient rats (Eisenstein and Hartroft, '57) but no change in its production by the adrenals of sodium deficient chickens (Taylor, Davis, Breitenbach and Hartroft, '70). The long term administration of sodium chloride brought about a slight atrophy of the peripheral and hypertrophy of the central cortical zone in the adrenal glands of pigeons (Kondics, '63). Kondics ('65) also observed that administration of prednisolone, a synthetic steroid which is thought to depress the secretion of ACTH by the adenohypophysis and the corticotropin releasing factor (CRF) by the median eminence, did not affect the histology of the peripheral interrenal cells but induced atrophy of the deeper cells of the pigeon adrenal gland. The administration of ACTH in pigeons caused hypertrophy of the interrenal cells of the central zone along with a diminution in the lipid content, although an increase in the dosage of ACTH also caused hypertrophy of the interrenal cells of the peripheral zone (Kondics, '65).

Kitchell and Wells ('52) hypophysectomized 20 day old rat fetuses by decapitation and observed the effects on the adrenal glands two to four days later. Hypophysectomy retarded the growth of the adrenal glands, decreased the width of the cortex and retarded the process of zonation. In the normal 20 day old rat fetus the adrenal cortex has two zones, an outer zone topographically similar to the zona glomerulosa of postnatal life and an inner zone topographically similar to the combined zona fasciculata and reticularis. In the normal 22 day old fetus, the adrenal cortex has three zones: an outer zone essentially like that seen at 20 days and an intermediate and inner

zone which were subdivisions of the inner zone seen at 20 days. The adrenal glands of 22 and 24 day old hypophysectomized fetuses lacked an intermediate zone and resembled the adrenals of normal 20 day old fetuses, the age at which hypophysectomy was performed.

At this time we are not aware of any studies on the effects of hypophysectomy on the development of the cortical zones in the adrenal gland of the chick embryo. However, should hypophysectomy affect the adrenal glands of the chick embryo in the same manner in which it affects the adrenal glands of rat fetuses, then the absence of histochemically demonstrable cortical zones in the adrenal glands of the former could be attributed to a failure of central zone development. The absence of cortical zones could conceivably also be attributed to an increase in the central zone P-3β-HSD activity level or a decrease in the central zone DHA-3β-HSD activity level to that of the peripheral zone. The presence of a smaller than normal central zone, with a higher than normal P-3β-HSD activity or a lower than normal DHA-3β-HSD activity could be the result of a retarded development of the central zone cortical tissue and infiltration of the central zone with peripheral zone tissue, or to a change in central zone 3β-HSD activity to the level of the peripheral zone, from periphery to center, thus reducing the size of the central zone.

Chick embryos hypophysectomized by means of partial decapitation, at about 40 hours of incubation not only lack the adenohypophysis and neurohypophysis but also the telencephalon, diencephalon and the anterior part of the mesencephalon. These embryos also lack eyes, nostrils, comb, and the upper beak which leaves the tongue exposed but does not affect the anterior

openings of the digestive and respiratory tract (fig. 19b). Occasionally the lower beak is absent (fig. 19c). Such embryos are stunted, and the ovaries, testes, thyroids and adrenal glands fail to develop to the extent seen in normal intact embryos. They also show a very poor survival rate. Betz ('67) has shown that most of the defects of chick embryos hypophysectomized by partial decapitation were due to the absence of the hormones of the pars distalis rather than to the absence of other areas of the brain. This investigator grafted adenohypophyses from ten day old, donor chick embryos onto the chorioallantoic membrane of nine day old hypophysectomized embryos and observed that the tropic hormones secreted by these grafts reduced the mortality of hypophysectomized embryos and enhanced their growth. The thyroids, gonads, and adrenal glands of such embryos when necropsied at the end of 20 days incubation, were in a near normal condition. The pars distalis grafts affected the development of these organs in partially decapitated embryos in the absence of the hypothalamus, epiphysis, pars nervosa and the prosencephalic and anterior mesencephalic derivatives of the brain. Tropic hormones from the grafted pars distalis were apparently synthesized and released in the absence of hypothalmic releasing factors.

In adult birds the adenohypophysis is under the control of releasing factors from the hypothalmus. Resko, Norton, and Nalbandov ('64) observed a decrease in the weight of adrenal glands and in the corticosterone concentration in the adrenal venous plasma of hypophysectomized immature white Leghorn cockerels, and in such cockerels with pituitary autografts, when compared to sham operated controls. Although the transplanted pituitaries appeared to be histologically normal, they did not secrete ACTH when removed

from the sella turcica. Electrolytic lesions in the hypothalamus of intact cockerels were found to cause a reduction in the corticosterone concentration in adrenal venous plasma (Frankel, et al., '67a). Dexamethasone is a synthetic analogue of cortisol which interfers with the ability of the anterior pituitary to secrete ACTH and has been reported to act at a hypothalamic or higher level (Corbin et al., '65). A small dose of dexamethasone phosphate was found to be effective in causing a complete inhibition of the adrenal corticosterone output following injections in intact domestic fowl (Frankel et al., 67b).

The near normal appearance of the adrenals of hypophysectomized chick embryos with pars distalis grafts (Betz, '67) showed that the production of ACTH in the chick embryo is independent of the hypothalamus and that the hypothalamic control system has not matured by ten days of incubation, the age of the donor embryos. In the present investigation the peripheral and central zones of the adrenal glands of chick embryos, hypophysectomized at approximately 40 hours, and with vascularized and well developed adenohypophyseal grafts, from 17 day old donor embryos, had about the same level of P- and DHA-3β-HSD activity as the zones of sham operated embryos of the corresponding age. Histologically, the adrenal glands of these embryos were indistinguishable from those of sham operated or normal embryos of the same age. Our results are in agreement with those of Betz ('67) in that they show that the production of ACTH in the chick embryo is independent of the hypothalamus but they also indicate that the hypothalamic control system has not matured by 17 days of incubation.

Woods, De Vries and Thommes ('71) observed that hypophysectomy of chick

embryos at 33 to 38 hours of incubation decreased the concentration of corticosteroids in the allantoic fluid beginning at 14.5 days. Pituitary transplants on the chorioallantoic membrane of such embryos restored the corticosteroid concentrations to values characteristic of intact embryos.

Bohus, Straznicky and Mess ('70) have shown that embryonic chick pituitary grafts deprived of their hypothalamic connections maintain their basal adenocorticotropic activity and are able to release ACTH in response to stimulation with metyrapone. The release of ACTH in response to the administration of the metyrapone is due to a negative feedback stimulus evoked by inhibiting adrenal 11β-hydroxylase, the enzyme which catalyzes the addition of the hydroxyl group at position 11 of the steroid nucleus in the biosynthetic pathway for corticosteroid formation.

In contrast to chick embryos, rat embryos require corticotropin-releasing factor (CRF) for the release of ACTH. Jost, Dupouy and Monchamp ('66) encephalectomized 19.5 day old rat fetuses in such a way that the pituitary gland was left in situ in the developing sella turcica. The adrenal gland weights of 21.5 day old encephalectomized fetuses were lower than those of intact controls and were about the same as the weights of decapitated fetuses of the same age. The administration of purified beef CRF or crude rat hypothalamic extracts prevented the effects of encephalectomy but had very little effect on fetuses lacking the pituitary. The adrenal glands of the encephalectomized or decapitated fetuses were not stimulated by maternal ACTH since this hormone apparently does not pass the placental barrier from the maternal to the fetal circulation (Picon, '57; Holland, '58).

The histological appearance of the adrenal glands of hypophysectomized embryos with adenohypophyseal transplants, appears to be related to the amount and the condition of the adenohypophyseal tissue and therefore, to the amount of ACTH being synthesized and released. When these transplants were vascularized and well developed, the adrenal glands were indistinguishable from those of sham operated or normal embryos. When the transplants contained only a small amount of adenohypophyseal tissue, or when this tissue was undergoing degeneration, the adrenal glands revealed many of the characteristics of those of non-treated hypophysectomized embryos, believed to be the result of a lower than normal production of ACTH.

The histological appearance and 3β-HSD activity in the adrenal glands of hypophysectomized embryos with transplants of brain tissue, were almost identical to those of non-treated hypophysectomized embryos. These observations indicate that the transplants were producing little or no ACTH or an ACTH-like substance. Salam, Norton and Nalbandov ('70) found no detectable ACTH or ACTH-like activity in extracts of chicken cerebral cortices.

The histological appearance of the adrenals of hypophysectomized embryos receiving ACTH appears to be related to the amount of ACTH administered. When such embryos received 1.0 IU of ACTH at 24 hour intervals, the adrenal glands appeared to be relatively normal through 12 days but after 12 days they revealed many of the characteristics of those of hypophysectomized embryos, indicating that the level of ACTH was not sufficiently high to maintain them in a normal condition. When these embryos received 1.0 IU of ACTH at 12 hour intervals (2.0 IU per day) the overall histological appearance of the adrenal glands was that of normal embryos, however, the peripheral

cortical cords were hypertrophied and the glands in some of the embryos were more vascular than normal. In no instance did the adrenals of these embryos resemble those of hypophysectomized embryos.

The results of the present investigation indicate that the level and distribution of central zone 3β-HSD activity, in the adrenal glands of hypophysectomized chick embryos, are also affected by the amount of ACTH administered. When these embryos received 1.0 IU of ACTH at 24 hour intervals, this activity was at levels characteristic of normal embryos from 10 through 12 days but after 12 days the activity was at levels characteristic of non-treated hypophysectomized embryos. When hypophysectomized embryos received 1.0 IU of ACTH at 12 hour intervals, the central zone 3β-HSD activity was characteristic of that of normal embryos through 18 days of incubation, the last stage examined.

Our observations of normal 3β-HSD activity levels and distribution, in the adrenal glands of treated hypophysectomized embryos, are supported by the work of Woods, DeVries and Thommes ('71) who reported that the decreased allantoic corticosteroid levels observed in hypophysectomized chick embryos, were restored to levels characteristic of normal embryos following ACTH administration or pituitary transplantation to the chorioallantoic membrane.

Levy, Deane and Rubin ('59) observed a reduction of 3β-HSD activity in the involuting inner cortical zones of hypophysectomized rats and by 50 days following hypophysectomy the inner zones had lost virtually all activity. Administration of ACTH for 8 days to rats hypophysectomized 70 days earlier resulted in hypertrophy and an increase in 3β-HSD activity in the inner cortical zones.

The results of our investigation indicate that the normal distribution and levels of histochemically demonstrable 3β-HSD activity, in the adrenal glands of the chick embryo, is dependent on the presence of the pituitary beginning on the eighth day of incubation. Removal of the pituitary gland resulted in a retardation or a failure in the development of the central cortical cords. Adenohypophyseal transplants, as well as the administration of ACTH, prevent development of the effects of hypophysectomy.

SUMMARY AND CONCLUSIONS

1 This investigation is based on a study of histochemically demonstrable 3β-hydroxysteroid dehydrogenase (3β-HSD) activity in the adrenal glands of 126 normal, 128 hypophysectomized, 83 sham operated and 118 treated hypophysectomized white and brown Leghorn embryos. An additional 44 normal, 22 hypophysectomized, 17 sham operated and 10 treated hypophysectomized embryos were used for histological examination of these glands.

2 A modification of the method of Levy, Deane and Rubin ('59) was used for the histochemical visualization of adrenal 3β-HSD activity. Dehydroepiandrosterone (DHA) and pregnenolone (P) were used as substrates for determining this activity. The activity was determined by a visual estimation of the density of diformazan deposition and was rated according to a 1 to 6 scale.

3 At stage 22, DHA- and P-3β-HSD activity were observed in the adrenocortical cells, proliferated from the peritoneal epithelium, which were located immediately dorsal to the epithelium. These activities increased steadily to eight days of incubation, however, the P-3β-HSD was always greater than the DHA-3β-HSD activity. Both activities appeared to be evenly distributed throughout the cortical cell groups and cords through stage 29.

4 On the eighth day, indications of a zonation were observed in the adrenal glands of some embryos. The P-3β-HSD activity was higher in the cortical

cords at the periphery of the gland than in those more centrally located. The distribution of DHA-3β-HSD activity was just the opposite, that is, it was greater in the central than in the peripheral cords. There is a close correlation between these observations, on the initial appearance of cortical zones at eight days, and those of other investigators who observed the onset of adrenocorticotropic activity of pituitary extracts and the initiation of corticoid secretion at eight days.

5 From nine days, and continuing through the remainder of the incubation period and the first 14 days after hatching, the adrenal glands showed a narrow peripheral cortical zone of high P-3β-HSD and low DHA-3β-HSD activity and a large central cortical zone of low P-3β-HSD and high DHA-3β-HSD activity. These differences in the intensity and distribution of P- and DHA-3β-HSD activity suggest the presence of two substrate-specific 3β-hydroxysteroid dehydrogenases.

6 The peripheral zone P-3β-HSD activity reached the maximum level of 6 between 10 and 12 days, then decreased somewhat until 19 days when it again reached the maximum level. During the first two weeks after hatching the activity remained at the maximum level.

7 The central zone P-3β-HSD activity was at its maximum level of 5 when this zone first appeared on the eighth day after which there was a steady decrease to a level of 3 by the time of hatching. This level was maintained during the first two weeks after hatching.

8 The peripheral zone DHA-3β-HSD activity increased slowly beginning at

eight days to a maximum level of 5 at the time of hatching which was maintained in the 14-day post-hatch period.

9 The central zone DHA-3β-HSD activity reached the maximum level of 6 at 12 days incubation and remained at this level throughout the remainder of the incubation period and during the post-hatch period.

10 The adrenal glands of some embryos were also examined for histochemically demonstrable reduced NAD:lipoamide oxidoreductase (diaphorase). The adrenal diaphorase activity was considerably greater than the DHA- or P-3β-HSD activity. After ten days of incubation the diaphorase activity was higher in the peripheral than in the central zone.

11 Hypophysectomy, by partial decapitation at stages 10 or 11 (33 to 45 hours), had no effect on the morphological development of the adrenal glands prior to ten days of incubation. After ten days the cortical cords of hypophysectomized embryos were hypertropied and less numerous than those of sham operated control or normal embryos.

12 Hypophysectomy had no effect on adrenal P- or DHA-3β-HSD activity prior to eight days of incubation. However, after eight days many of the adrenal glands of hypophysectomized embryos lacked cortical zones and when these did occur the peripheral zone was wider and the central zone smaller than those of controls.

13 From eight through 19 days, the central zone P-3β-HSD activity of hypophy-

sectomized embryos was higher and from 12 through 19 days the peripheral zone activity was lower than that of controls. After 12 days, central zone DHA-3β-HSD activity was lower while that of peripheral zone was equal to controls from eight through 19 days. The P- and DHA-3β-HSD levels, of the non-zonated adrenal glands of hypophysectomized embryos, were about equal to the peripheral zone P- and DHA-3β-HSD levels of the zonated adrenals of these embryos.

14 The transplantation of single, whole adenohypophyses from 17 day donor embryos to the chorioallantoic membrane of hypophysectomized 8.5 day embryos, resulted in normal development of the adrenal gland. The distribution and levels of DHA- and P-3β-HSD activity were characteristic of embryos with intact pituitaries.

15 The administration of ACTH in hypophysectomized embryos resulted in normal development of the adrenal glands. The distribution and levels of DHA- and P-3β-HSD activity were characteristic of embryos with intact pituitaries.

16 It is concluded that the adrenocortical cells of the chick embryo have a steroidogenic potential as soon as they can be recognized early in development. Development of the adrenal glands prior to the eighth day is independent of adenohypophyseal control. However, the appearance of cortical zones is, at least partially, dependent on the production of ACTH by the adenohypophysis. The absence of the pituitary gland results in either a retarded development, or a failure in development of the

central cortical zone. Adenohypophyseal transplants, as well as administration of ACTH, inhibit development of the effects of hypophysectomy.

17 It is also concluded that hypothalamic control of the release of ACTH by the adenohypophysis in the chick embryo, has not developed by 17 days of incubation.

LITERATURE CITED

Adjovi, Yvette 1970 Morphogenèse et activitè de la glands cortico-surrénale de l'embryon de poulet normal et décapité. Arch. Anat. Micro. Morph. Exp., 59: 185-200.

Arrington, L. R., M. H. Fox and H. A. Bern 1952 Androgen content of testis and adrenal of white Leghorn cockerels. Endocrinology, 51: 226-236.

Arvy, L. 1961 Contribution à l'histochimie de la glande surrénale chez Gallus domesticus et chez Anas boschas L. C. R. Soc. Biol., 155: 69-71.

_____ 1962 Presence d'une activite steroido-3β-ol-deshydrogenasique chez Sauropsides. C. R. Acad. Sci., 255: 1803-1804.

Ashbel, R., and A. M. Seligman 1949 A new reagent for the histochemical demonstration of active carbonyl groups. A new method for staining ketonic steroids. Endocrinology, 44: 565-583.

Baillie, A. H., M. M. Ferguson and D. McK. Hart 1966 Developments in Steroid Histochemistry. Academic Press, New York.

Baillie, A. H., and K. Griffiths 1964 3β-Hydroxysteroid dehydrogenase in the foetal mouse Leydig cell. J. Endocr., 31: 63-66.

Betz, T. W. 1967 The effects of embryonic pars distalis grafts on the development of hypophysectomized chick embryos. Gen. Comp. Endocr., 9: 172-186.

Bloch, E., K. Benirschke and E. Rosemberg 1956 C_{19}-Steroids, 17α-OH-corticosterone and a sodium retaining factor in human fetal adrenal glands. Endocrinology, 58: 626-633.

Bloch, E., R. I. Dorfman and G. Pincus 1956 The conversion of acetate to dehydroepiandrosterone by human adrenal gland slices. Arch. Biochem. Biophys., 61: 245-247.

Bloch, E., G. Pincus and R. I. Dorfman 1956 Conversion of acetate to C_{19} steroids by human adrenal gland slices. Fed. Proc., 15: 222.

Bohus, B., K. Straznicky and B. Mess 1970 ACTH release from hetertropic pituitary grafts in chicken embryos treated with metyrapone. Acta Physiol. Acad. Sci. Hung., 37: 259-264.

Bongiovanni, A. M., and A. W. Root 1964 The adrenogenital syndrome. New Eng. J. Med., 268: 1283-1289, 1342-1351, 1391-1399.

Bonhommet, M., and J.-P. Weniger 1967 Sécrétion de corticostéroides par la surrénale embryonnaire de Poulet, cultivée in vitro. C. R. Soc. Biol., 161: 2052-2055.

Boucek, R. J., Eva Györi and R. Alvarez 1966 Steroid dehydrogenase reactions in developing chick adrenal and gonadal tissues. A histochemical study. Gen. Comp. Endocr., 7: 292-303.

Case, J. F. 1951 Influence of pituitary on adrenal chemodifferentiation in the chick embryo. Anat. Rec., 109: 277-278.

_____ 1952 Adrenal cortical-anterior pituitary relationships during embryonic life. Ann. N.Y. Acad. Sci., 55: 147-158.

Castañé Decoud, A., E. Pedernera and R. Narbaitz 1964 Accion del ACTH sobre la suprarrenal del embrion de pollo. Rev. Soc. Argent. Biol., 40: 181-186.

Chayen, J., L. Bitensky, R. G. Butcher and L. W. Poulter 1969 A Guide to Practical Histochemistry. J. B. Lippincott Company, Philadelphia. pp. 207-208.

Chieffi, G., H. Manelli, V. Botte and L. Mastrolia 1964 Osservazioni sul precoce differenziamento funzionale dell'interrenale nell'embrione di pollo. Atti Accad. Naz. Lincei, Ser. 8, 37: 82-85.

Corbin, A., G. Mangili, M. Motta and L. Martini 1965 Effect of hypothalamic and mesencephalic steroid implantations on ACTH feedback mechanism. Endocrinology, 76: 811-818.

Dawson, D. B. 1953 Histochemical evidence of early differentiation of the suprarenal gland of the chick. J. Morph., 92: 579-596.

Deane, H. W. 1962 The anatomy, chemistry, and physiology of adrenocortical tissue. In: Handbuch der Experimentellen Pharmakologie, Vol. 14, pt. 1, The Adrenocortical hormones: Their origin, chemistry, physiology, and Pharmacology. Springer-Verlag, Berlin. pp. 1-185

Deane, H. W., and R. O. Greep 1946 A morphological and histochemical study of the rat's adrenal cortex after hypophysectomy, with comments on the liver. Am. J. Anat., 79: 117-145.

Deane, H. W., J. H. Shaw and R. O. Greep 1948 The effect of altered sodium or potassium intake on the width and cytochemistry of the zona glomerulosa of the rat's adrenal cortex. Endocrinology, 43: 133-153.

deRoos, R. 1961 The corticoids of the avian adrenal gland. Gen. Comp. Endocr., 1: 494-512.

deRoos, R., and C. C. deRoos 1964 Effects of mammalian corticotropin and chicken adenohypophysial extracts on steroidogenesis by chicken adrenal tissue in vitro. Gen. Comp. Endocr., 4: 602-607.

Domm, L. V., and G. C. Ericson 1972 Histochemical localization of steroidogenic sites in the rudimentary right gonad of the female chick embryo. IV Intern. Congr. Endocr., Washington, D.C.

Donaldson, E. M., W. N. Holmes and J. Stachenko 1965 In vitro corticosteroidogenesis by the duck (Anas platyrhynchos) adrenal. Gen. Comp. Endocr., 5: 542-551.

Dorfman, R. I., and D. C. Sharma 1965 An outline of the biosynthesis of corticosteroids and androgens. Steroids, 6: 229-236.

Eisenstein, A. B., and P. M. Hartroft 1957 Alterations in the rat adrenal cortex by sodium deficiency: steroid hormone secretion. Endocrinology, 60: 634-640.

Ericson, G. C. 1968 The effects of exogenous ACTH on Δ^5-3β-hydroxysteroid dehydrogenase activity in the embryonic avian adrenal gland. Masters Thesis, Loyola University Stritch School of Medicine, Maywood, Illinois.

Ericson, G. C., and L. V. Domm 1969 Δ^5-3β-Hydroxysteroid dehydrogenase activity in the embryonic adrenal gland of the Leghorn domestic fowl. Anat. Rec., 163: 300-301.

Fortier, C. 1959 Note on the extraction and storage of the rat pituitary prior to in vitro assay for ACTH. Canad. J. Biochem. Physiol., 37: 608-611

Frankel, A. I. 1970 Neurohumoral control of the avian adrenal: A review. Poultry Sci., 49: 869-921.

Frankel, A., J. W. Graber and A. V. Nalbandov 1967a The effect of hypothalamic function in intact and adenohypophysectomized cockerels. Gen. Comp. Endocr., 8: 387-396.

_____ 1967b Adrenal function in cockerels. Endocrinology, 80: 1013-1019.

Fugo, N. W. 1940 Effects of hypophysectomy in the chick embryo. J. Exp. Zool., 85: 271-297.

Greep, R. O., and H. W. Deane 1949 The cytology and cytochemistry of the adrenal cortex. Ann. N.Y. Acad. Sci., 50: 596-615.

Hall, P. F., and S. B. Koritz 1966 Action of ACTH upon steroidogenesis in the chicken adrenal gland. Endocrinology, 79: 652-654.

Hamburger, V., and H. L. Hamilton 1951 A series of normal stages in the development of the chick embryo. J. Morph., 88: 49-92.

Hamilton, H. L. 1952 Lillie's Development of the Chick. Henry Holt and Company, New York. pp. 70-91.

Hart, D. McK., A. H. Baillie, K. C. Calman and M. M. Ferguson 1966 Hydroxysteroid dehydrogenase in mouse adrenal and gonads. J. Anat., 100: 801-812.

Hays. V. J. 1914 The development of the adrenal glands of birds. Anat. Rec., 8: 451-474.

Hinni, J. B., and R. L. Watterson 1963 Modified development of the duodenum of chick embryos hypophysectomized by partial decapitation. J. Morph., 113: 381-425.

Holland, R. C. 1958 The effect of hypoxia on the fetal rat adrenal. Anat. Rec., 130: 177-195.

Jost, A., J.-P. Dupouy and A. Monchamp 1966 Fonction corticotrope de l'hypophyse et hypothalamus chez le foetus de rat. C. R. Acad. Sci., 262: 147-150.

Kar, A. B. 1947 The adrenal cortex testicular relations in the fowl: The effect of castration and replacement therapy on the adrenal cortex. Anat. Rec., 99: 177-197.

Kitchell, R. L., and L. J. Wells 1952 Functioning of the hypophysis and adrenals in fetal rats: effects of hypophysectomy, adrenalectomy, castration, injected ACTH and implanted sex hormones. Anat. Rec., 112: 561-591.

Kjaerheim, A. 1968 Studies on adrenocortical ultrastructure. 4. Effects of ACTH on interrenal cells of the domestic fowl. J. Microscopie, 7: 715-738.

Kondics, L. 1963 Uber die Wirkung des Kochsalzes auf die interrenalen Zellen der Nebenniere bei Haustauben. Ann. Univ. Sci. Budapest Rolando Eotovos Nominatae, Sect. Biol., 6: 101-107.

_____ 1965 Die Wirkung von ACTH und Prednisolone auf die funktionale Zonation der Nebenniere bei der Taube (Columba domestica). Acta Morph. Acad. Sci. Hung., 13: 233-240.

Kondics, L., and A. Kjaerheim 1966 The zonation of interrenal cells in fowls (An electron microscopical study). Z. Zellforsch., 70: 81-90.

Levy, H., H. W. Deane and B. L. Rubin 1959 Visualization of steroid-3β-ol-dehydrogenase activity in tissues of intact and hypophysectomized rats. Endocrinology, 65: 932-943.

Lillie, R. D. 1966 Histopathologic Technic and Practical Histochemistry. McGraw-Hill Book Company, New York. pp. 472-474.

Lobel, B. L., H. W. Deane and S. L. Romney 1962 Enzymic histochemistry of the villous portion of the human placenta from six weeks of gestation to term. Am. J. Obst. Gynec., 83: 295-299.

Manelli, H. 1964 Ipofisectomia e differenziamento funzionale delle surrenali e delle gonadi embrionali di pollo: reazione della Δ^5-3β-idrossisteroide deidrogenasi. Boll. Zool., 31: 555-558.

Manelli, H., and L. Mastrolia 1963 Azione dell'ACTH e reazione della steroide 3β-olodeidrogenasi nelle surrenali embrionali de pollo coltivate in vitro. Atti Accad. Naz. Lincei, Ser. 8, 35: 598-601.

Mastrolia, L., and H. Manelli 1967 Localizzazione istochimica della glucoso-6-fosfato e 6-fosfogluconico deidrogenasi e loro significato metabolico nelle surrenali dell'embrione e dell'adulto di Pollo. Arch. Zool. Ital., 52: 237-244.

McGowen, J. P. 1936 Suprarenal virilism in a domestic hen: Its possible significance. J. Exp. Biol., 13: 377.

Miller, R. A. 1967 Regional responses of interrenal tissue and of chromaffin tissue to hypophysectomy and stress in pigeons. Acta Endocr., 55: 108-118.

Miller, R. A., and O. Riddle 1942 The cytology of the adrenal cortex of normal pigeons and in experimentally induced atrophy and hypertrophy. Am. J. Anat., 71: 311-342.

Minoura, T. 1921 A study of testis and ovary grafts on the hen's egg and their effects on the embryo. J. Exp. Zool., 33: 1-61.

Moog, F. 1959 The development of function in the adrenal cortex. In: Comparative Endocrinology. A. Gorbman, Ed. John Wiley and Sons, Inc., New York. pp. 624-638.

_____ 1961 The functional differentiation of the small intestine. IX. The influence of thyroid function on cellular differentiation and accumulation of alkaline phosphatase in the duodenum of the chick embryo. Gen. Comp. Endocr., 1: 416-432.

Moog, F., and E. Ford 1957 Influence of exogenous ACTH on body weight, adrenal growth, duodenal phosphatase, and liver glycogen in the chick

embryo. Anat. Rec., 128: 592.

Moog, F., and D. Richardson 1955 The functional differentiation of the small intestine. IV. The influence of adrenocortical hormones on differentiation and phosphatase synthesis in the duodenum of the chick embryo. J. Exp. Zool., 130: 29-55.

Moog, F., and E. R. Thomas 1957 Functional differentiation of the small intestine. VI. Transient accumulation of glycogen in intestinal epithelium of chick embryo under normal conditions and under influence of hydrocortisone. Physiol. Zool., 30: 281-287.

Nagra, C. L., J. G. Birnie, G. J. Baum and R. K. Meyer 1963 The role of the pituitary in regulating steroid secretion by the avian adrenal. Gen. Comp. Endocr., 3: 274-280.

Narbaitz, R., and M. T. Sabatini 1963 Histochemical demonstration of cholesterol in differentiating chick gonads. Z. Zellforsch., 59: 1-5.

Pedernera, E. A. 1968 Differentiation in vitro of chick embryo adrenal glands. Experientia, 24: 938-939.

_____ 1971 Development of the secretory capacity of the chick embryo adrenal glands. J. Embry. Exp. Morph., 25: 213-222.

Picon, L. O. 1957 Influence de l'hyperfonctionnement des surrénales maternelles sur le developpement des surrénales foetales chez le rat. C. R. Soc. Biol., 151: 1104-1106.

Rawles, Mary E. 1952 Transplantation of normal embryonic tissues. Ann. N.Y. Acad. Sci., 55: 302-312.

Resko, J. A., H. W. Norton and A. V. Nalbandov 1964 Endocrine control of the adrenal in chickens. Endocrinology, 75: 192-200.

Roberts, S., and C. M. Szego 1955 Biochemistry of steroid hormones. Ann. Rev. Biochem., 24: 543-596.

Romanoff, A. L. 1960 The Avian Embryo: Structural and Functional Development. The Macmillan Company, New York.

Salem, M. H. M., H. W. Norton and A. V. Nalbandov 1970 A study of ACTH and CRF in chickens. Gen. Comp. Endocr., 14: 270-280.

Samuels, L. T., and K. B. Eik-Nes 1968 Metabolism of steroid hormones. In: Metabolic Pathways, 3rd edition, Vol. 2. D. M. Greenberg, Ed. Academic Press, N.Y. pp. 169-220.

Samuels, L. T., and M. L. Helmreich 1956 The influence of chorionic gonadotropin on the 3 -ol dehydrogenase activity of testes and adrenals. Endocrinology, 58: 435-442.

Samuels, L. T., and T. Uchikawa 1967 Biosynthesis of adrenal steroids. In: The Adrenal Cortex. A. B. Eisenstein, Ed. Little, Brown and Company Boston. pp. 61-102.

Sandor, T. 1969 A detailed study of the biogenesis of corticosteroids in the avian adrenal by in vitro techniques. In: Progress in Endocrinology Proc. 3rd Intern. Congr. Endocr. Excerpta Medica Intern. Congr. Ser. No. 184. pp. 730-737.

Sandor, T., J. Lamoureux and A. Lanthier 1963 Adrenocortical function in birds: In vitro biosynthesis of radioactive corticosteroids from pregnenolone-7-H^3 and progesterone-4-C^{14} by adrenal glands of the domestic duck (Anas platyrhynchos) and the chicken (Gallus domesticus). Endocrinology, 73: 629-636.

_____ 1965 Adrenal cortical function in birds. The in vitro transformation of sodium acetate-1-^{14}C and cholesterol-4-^{14}C by adrenal gland preparations of the domestic duck (Anas platyrhynchos) and the goose (Anser anser). Steroids, 6: 143-157.

Sandor, T., and A. Lanthier 1963 The biosynthesis in vitro of radioactive corticosteroids from (4-^{14}C) progesterone by adrenal slices of the domestic duck (Anas platyrhynchos). Biochim. Biophys. Acta, 74: 756-762.

Schultz, A. 1924 Eine methode des mikrochemischen Cholesterinnachweises am Gewebsschnitt. Zentr. Allgem. Pathol. Anat., 35: 314-317.

Short, R. V. 1960 The secretion of sex hormones by the adrenal gland. Biochem. Soc. Symp., 18: 59-84.

Sivaram, Saraswati 1964 Histochemistry of the developing adrenal gland of the chick. Anat. Rec., 148: 336.

_____ 1965 Structure and development of the adrenal gland of Gallus domesticus. Canad. J. Zool., 43: 1021-1031.

_____ 1968 Histochemical studies on the developing adrenal gland of Gallus domesticus. Histochemie, 12: 316-325.

_____ 1969 Nature and distribution of lipids in the developing adrenal cortex of Gallus domesticus. Acta Histochem., 32: 253-261.

Straznicky, K., F. Hajos and B. Bohus 1966 Relationship between the ultrastructure and cortical activity of the embryonic adrenal gland in the

chicken. Acta Biol. Hung., 16: 261-274.

Sturkie, P. D. 1965 Avian Physiology, 2nd ed. Comstock Publishing Associates New York. pp. 534-567.

Székely, Gy., E. Endróczi and J. Szentagothai 1958 Erscheinen der argentaffinen substanz und beginn der sekretion adrenocorticotrophen hormons in der hypophyse von huhnerembryonen. Acta Biol. Hung., 8: 283-287.

Taylor, A. A., J. O. Davis, R. P. Breitenbach and P. M. Hartroft 1970 Adrenal steroid secretion and a renal-pressor system in the chicken (Gallus domesticus). Gen. Comp. Endocr., 14: 321-333.

Toth, N., P. Simon and Gy. Székely 1958 Abhangigkeit der fruhen geweblichen Differentiation der Nebennierenrinde von der Hypophyse bei Huhnerembryonen. Acta Biol. Acad. Sci. Hung., 8: 289-294.

Wattenberg, L. W. 1958 Microscopic histochemical demonstration of steroid-3β-ol dehydrogenase in tissue sections. J. Histochem. Cytochem., 6: 225-232.

Watterson, R. L., D. A. Brown and A. Bartha 1959 Reduced endocrine activity of the adrenal cortex of hypophysectomized chick embryos as indicated by modified development of the duodenum. Anat. Rec., 133: 347-348.

Willier, B. H. 1924 The endocrine glands and the development of the chick. I. The effects of thyroid grafts. Am. J. Anat., 33: 67-103.

Woods, J. E., and L. V. Domm 1966 A histochemical identification of the androgen-producing cells in the gonads of the domestic fowl and albino rat. Gen. Comp. Endocr., 7: 559-570.

Woods, J. E., G. W. DeVries and R. C. Thommes 1971 Ontogenesis of the pituitary-adrenal axis in the chick embryo. Gen. Comp. Endocr., 17: 407-415.

Woods, J. E., and R. L. Weeks 1969 Ontogenesis of the pituitary-gonadal axis in the chick embryo. Gen. Comp. Endocr., 13: 242-254.

Zarrow, M. X., D. L. Greenman, J. Kollias and D. Dalrymble 1962 The pituitary adrenal axis in the bird. Gen. Comp. Endocr., 2: 177-180.

TABLE 1

THE NUMBER OF EMBRYOS EMPLOYED THROUGHOUT THE INVESTIGATION

	Total number	Number alive at necropsy	Number frozen sectioned	Number paraffin sectioned
Normals	194	194	126	44
Sham operated embryos	349	131	83	17
Hypophysectomized embryos	528	128	103	22
H+A embryos[1]	60	34	28	6
H+B embryos[2]	30	11	11	0
H+ACTH embryos[3]	77	63	59	4
H+VEH embryos[4]	25	20	20	0
Donors[5]	60	-	-	-
Totals	1323	581	430	93

[1] Hypophysectomized embryos with adenohypophyseal transplants.
[2] Hypophysectomized embryos with transplants of small pieces of brain.
[3] Hypophysectomized embryos in which ACTH was administered.
[4] Hypophysectomized embryos which received hormone vehicle.
[5] Embryos used as donors for adenohypophyseal transplants.

TABLE 2

EMBRYOS PROCESSED FOR HISTOCHEMICAL AND HISTOLOGICAL EXAMINATION

Age		Experiment I		Experiment II			
Embryos		Normal		Hypophysectomized		Sham Operated	
Stage	Days	3β-HSD[1]	Histo.[2]	3β-HSD	Histo.	3β-HSD	Histo.
21	3½	0	5	1	0	0	0
22	3½-4	4	4	2	0	0	1
23	4	5	6	4	3	3	1
24	4½	5	3	5	2	1	0
25	4½-5	7	3	4	2	5	2
26	5	4	3	6	1	2	1
27	5-5½	7	2	4	0	2	0
28	5½-6	5	5	2	1	1	1
29	6-6½	8	1	5	0	1	0
30	6½-7	4	2	4	0	4	0
32	7½	7	1	0	0	0	0
34	8	5	1	6	0	8	0
35	9	11	0	6	2	6	1
36	10	13	1	6	1	7	2
37	11	7	1	5	2	5	1
38	12	2	1	6	1	8	1
39	13	1	0	2	1	2	0
40	14	5	0	4	2	6	1

TABLE 2 (cont'd)

Age		Experiment I		Experiment II			
Embryos		Normal		Hypophysectomized		Sham Operated	
Stage	Days	3β-HSD[1]	Histo.[2]	3β-HSD	Histo.	3β-HSD	Histo.
41	15	1	2	9	1	6	2
42	16	6	1	5	2	3	1
43	17	5	2	7	1	4	2
44	18	6	0	5	0	3	0
45	19	1	0	4	0	4	0
46	20-21	7	0	1	0	2	0
Chicks							
Days							
1		3	0				
3		1	0				
7		1	0				
14		1	0				
Total Processed		132	44	103	22	83	17

[1] Number processed for histochemical determinations.

[2] Number processed for histological examination.

TABLE 3

EMBRYOS PROCESSED FOR HISTOCHEMICAL AND HISTOLOGICAL EXAMINATION

Age		Experiment III				Experiment IV			
		H+A Embryos[1]		H+B Embryos[2]		H+ACTH Embryos[3]		H+VEH Embryos[4]	
Stage	Days	3β-HSD[5]	Histo[6]	3β-HSD	Histo.	3β-HSD	Histo.	3β-HSD	Histo.
35	9	2	0	0	0	4	0	3	0
36	10	3	2	3	0	5	2	4	0
37	11	3	0	2	0	5	1	1	0
38	12	3	1	0	0	7	0	3	0
39	13	2	1	1	0	9	0	3	0
40	14	2	1	2	0	10	0	1	0
41	15	3	1	0	0	6	1	2	0
42	16	1	0	0	0	2	0	1	0
43	17	6	0	2	0	4	0	1	0
44	18	1	0	1	0	4	0	0	0
45	19	2	0	0	0	3	0	1	0
Total Number Processed		28	6	11	0	59	4	20	0

[1] Hypophysectomized embryos with adenohypophyseal transplants.

[2] Hypophysectomized embryos with transplants of small pieces of brain.

[3] Hypophysectomized embryos which received ACTH.

[4] Hypophysectomized embryos which received hormone vehicle.

[5] Number processed for histochemical determinations.

[6] Number processed for histological examination.

TABLE 4

EVALUATION OF 3β-HSD ACTIVITY IN ADRENAL GLANDS OF NORMAL EMBRYOS AND CHICKS

Age		Average Adrenal 3β-HSD Activity[1]								
Embryos		P-3β-HSD				DHA-3β-HSD				
Stage	Days	Non-zonated	Peripheral Zone		Central Zone	Non-zonated	Peripheral Zone		Central Zone	
22	3½-4	Trace(4)[2]				Trace(4)				
23	4	2.0 (5)				1.0 (5)				
24	4½	2.8 (5)				2.0 (5)				
25	4½-5	3.7 (7)				2.7 (7)				
26	5	4.1 (4)				2.7 (4)				
27	5-5½	4.1 (7)				3.1 (7)				
28	5½-6	4.1 (5)				3.0 (5)				
29	6-6½	5.5 (8)				4.2 (8)				
30	6½-7	5.7 (3)	6.0	(1)	5.0	4.3 (3)	4.0	(1)	5.0	
32	7½	5.7 (5)	6.0	(2)	4.7	4.6 (7)		(0)		
34	8	6.0 (2)	5.3	(3)	5.0	5.0 (2)	4.5	(3)	5.3	
35	9		5.5	(11)	4.6	5.2 (3)	4.0	(8)	5.2	
36	10		5.8	(13)	4.3	5.0 (3)	4.3	(10)	5.3	
37	11		5.9	(7)	4.0		4.4	(7)	5.3	
38	12		6.0	(2)	4.7		5.0	(2)	6.0	
39	13		5.5	(1)	3.0		5.0	(1)	6.0	
40	14		5.8	(5)	4.4	5.5 (1)	4.8	(4)	5.6	
41	15		5.0	(1)	3.5		5.0	(1)	6.0	

TABLE 4 (cont'd)

Age		Average Adrenal 3β-HSD Activity[1]							
Embryos		P-3β-HSD				DHA-3β-HSD			
Stage	Days	Non-zonated	Peripheral Zone		Central Zone	Non-zonated	Peripheral Zone		Central Zone
42	16		5.3	(6)	3.5		5.0	(6)	6.0
43	17		5.1	(5)	3.3		4.3	(5)	6.0
44	18		5.0	(6)	3.1		5.0	(6)	5.7
45	19		6.0	(1)	3.0		5.5	(1)	6.0
46	20-21		6.0	(7)	3.1	6.0 (2)	5.3	(5)	5.9
Chicks									
Days									
1			5.7	(3)	3.1	6.0 (1)	5.5	(2)	6.0
3			6.0	(1)	3.0		4.5	(1)	5.0
7			6.0	(1)	3.0		5.5	(1)	6.0
14			6.0	(1)	3.0		5.5	(1)	6.0

[1] Average values of density of diformazan deposition.

[2] Numbers in parenthesis indicate number of animals.

TABLE 5

EVALUATION OF P-3β-HSD ACTIVITY IN ADRENAL GLANDS OF HYPOPHYSECTOMIZED AND SHAM OPERATED EMBRYOS

Age		Average Adrenal P-3β-HSD Activity[1]					
		Hypophysectomized			Sham Operated		
Stage	Days	Non-zonated	Peripheral Zone	Central Zone	Non-zonated	Peripheral Zone	Central Zone
22	3½-4	Trace(2)[2]					
23	4	2.4 (4)			2.3 (3)		
24	4½	2.5 (5)			3.5 (1)		
25	4½-5	3.8 (4)			3.5 (5)		
26	5	4.1 (6)			2.7 (2)		
27	5-5½	4.9 (4)			4.5 (2)		
28	5½-6	5.5 (2)			5.0 (1)		
29	6-6½	5.4 (5)			5.0 (1)		
30	6½-7	5.2 (4)			5.8 (4)		
34	8	5.5 (3)	5.8 (3)	5.2	5.9 (4)	5.7 (4)	4.9
35	9	5.8 (5)	5.5 (1)	5.0		5.6 (6)	4.6
36	10		5.8 (6)	5.3		6.0 (7)	4.8
37	11		5.9 (5)	5.1		6.0 (5)	4.5
38	12	5.5 (3)	5.3 (3)	4.8		6.0 (8)	4.4
39	13	6.0 (1)	5.0 (1)	4.5		5.7 (2)	4.2
40	14	5.7 (1)	5.6 (3)	4.6		5.7 (6)	4.2
41	15	5.5 (2)	5.1 (7)	4.6		5.8 (6)	4.1

TABLE 5 (cont'd)

Age		Average Adrenal P-3β-HSD Activity								
		Hypophysectomized				Sham Operated				
Stage	Days	Non-zonated	Peripheral Zone		Central Zone	Non-zonated	Peripheral Zone		Central Zone	
42	16	5.0 (1)	5.2	(4)	4.5		5.3	(3)	4.1	
43	17	5.0 (1)	5.8	(6)	4.6		5.5	(4)	3.5	
44	18		5.5	(5)	4.6		5.0	(3)	3.8	
45	19		5.1	(4)	4.4		5.7	(4)	3.4	

[1] Average values of density of diformazan deposition.

[2] Numbers in parenthesis indicate number of embryos.

TABLE 6

EVALUATION OF DHA-3β-HSD ACTIVITY IN ADRENAL GLANDS OF HYPOPHYSECTOMIZED AND SHAM OPERATED EMBRYOS

Age		Average Adrenal DHA-3β-HSD Activity[1]							
		Hypophysectomized			Sham Operated				
Stage	Days	Non-zonated	Peripheral Zone	Central Zone	Non-zonated	Peripheral Zone	Central Zone		
22	3½-4	Trace(2)[2]							
23	4	1.1 (4)			1.5 (3)				
24	4½	1.6 (5)			2.0 (1)				
25	4½-5	2.6 (4)			2.4 (5)				
26	55	2.8 (6)			1.7 (2)				
27	5-5½	3.0 (4)			3.5 (2)				
28	5½-6	4.0 (2)			4.0 (1)				
29	6-6½	4.0 (5)			4.0 (1)				
30	6½-7	4.1 (4)			4.3 (4)				
34	8	4.5 (5)	4.0	(1)	5.5	5.0 (6)	4.5	(2)	5.0
35	9	5.0 (5)	4.0	(1)	5.5	5.2 (2)	4.4	(4)	5.5
36	10	5.0 (1)	4.6	(5)	5.1		4.0	(7)	5.4
37	11		4.8	(5)	5.4		4.2	(5)	5.0
38	12	5.0 (1)	4.6	(5)	5.4		4.8	(8)	5.6
39	13	5.0 (1)	4.5	(1)	5.0		5.0	(2)	6.0
40	14		5.0	(4)	5.5	5.0 (1)	4.9	(5)	5.6
41	15	4.5 (1)	4.7	(8)	5.0		4.8	(6)	5.7

TABLE 6 (cont'd)

Age		Average Adrenal DHA-3β-HSD Activity[1]					
		Hypophysectomized			Sham Operated		
Stage	Days	Non-zonated	Peripheral Zone	Central Zone	Non-zonated	Peripheral Zone	Central Zone
42	16		4.6 (5)	5.0		4.3 (3)	5.3
43	17	5.5 (4)	4.5 (3)	4.7	5.0 (1)	4.6 (3)	5.7
44	18		5.2 (5)	5.1		4.5 (3)	5.2
45	19	5.5 (1)	4.8 (3)	4.7	5.5 (1)	5.0 (3)	5.7

[1] Average values of density of diformazan deposition.

[2] Numbers in parenthesis indicate number of embryos.

TABLE 7

EVALUATION OF P-3β-HSD ACTIVITY IN ADRENAL GLANDS OF HYPOPHYSECTOMIZED EMBRYOS WITH ADENOHYPOPHYSEAL AND BRAIN TRANSPLANTS

Age		Average Adrenal P-3β-HSD Activity[1]					
		H+A Embryos[2]			H+B Embryos[3]		
Stage	Days	Non-zonated	Peripheral Zone	Central Zone	Non-zonated	Peripheral Zone	Central Zone
35	9		5.5 (2)[4]	5.0			
36	10		5.7 (2)	4.6	6.0 (1)	5.2 (2)	4.5
37	11		6.0 (3)	5.0		5.5 (2)	4.7
38	12		6.0 (3)	4.2			
39	13		6.0 (2)	4.2		6.0 (1)	5.0
40	14		5.7 (2)	3.0	6.0 (1)	5.5 (1)	5.0
41	15		5.8 (3)	4.2			
42	16		5.0 (1)	3.5			
43	17		5.4 (6)	4.0	5.7 (2)		
44	18		5.0 (1)	3.0		5.0 (1)	4.0
45	19		5.7 (2)	3.2			

1 Average values of density of diformazan deposition.

2 Hypophysectomized embryos with adenohypophyseal transplants.

3 Hypophysectomized embryos with transplants of small piece of brain.

4 Numbers in parenthesis indicate number of embryos.

TABLE 8

EVALUATION OF DHA-3β-HSD ACTIVITY IN ADRENAL GLANDS OF HYPOPHYSECTOMIZED EMBRYOS WITH ADENOHYPOPHYSEAL AND BRAIN TRANSPLANTS

Age		Average Adrenal DHA-3β-HSD Activity[1]							
		H+A Embryos[2]			H+B Embryos[3]				
Stage	Days	Non-zonated	Peripheral Zone		Central Zone	Non-zonated	Peripheral Zone		Central Zone
35	9		4.2	(2)[4]	5.0				
36	10	5.0 (1)	4.5	(2)	4.7	5.0 (1)	4.2	(2)	5.0
37	11	5.0 (1)	4.7	(2)	5.5		4.5	(2)	5.0
38	12		4.5	(3)	5.5				
39	13		5.0	(2)	5.5		4.0	(1)	5.0
40	14	5.5 (1)	5.0	(1)	5.5		4.7	(2)	5.7
41	15	6.0 (1)	5.5	(2)	6.0				
42	16		4.5	(1)	5.0				
43	17		4.7	(6)	5.6	4.5 (2)			
44	18		4.0	(1)	5.5		4.0	(1)	5.5
45	19		5.7	(2)	5.0				

[1] Average values of density of diformazan deposition.

[2] Hypophysectomized embryos with adenohypophyseal transplants.

[3] Hypophysectomized embryos with transplants of small piece of brain.

[4] Numbers in parenthesis indicate number of embryos.

TABLE 9

EVALUATION OF DHA- AND P-3β-HSD ACTIVITY IN ADRENAL GLANDS OF HYPOPHYSECTOMIZED EMBRYOS WHICH RECEIVED ACTH AT 12 HOUR INTERVALS

Age		Average Adrenal 3β-HSD Activity[1]					
		P-3β-HSD			DHA-3β-HSD		
Stage	Days	Non-zonated	Peripheral Zone	Central Zone	Non-zonated	Peripheral Zone	Central Zone
35	9		6.0 (2)[2]	5.2	5.5 (1)	4.5 (1)	5.0
36	10		6.0 (2)	5.2		5.0 (2)	5.5
37	11		5.5 (2)	4.0	5.0 (2)	(0)	
38	12		5.8 (3)	4.5	5.3 (3)	(0)	
39	13		5.9 (4)	4.1		4.7 (4)	5.2
40	14		5.4 (5)	4.2	4.5 (4)	5.0 (1)	6.0
41	15		5.7 (3)	4.5	4.5 (1)	4.5 (2)	5.0
42	16		5.0 (2)	4.2	4.5 (2)	(0)	
43	17		5.5 (2)	4.0	5.5 (1)	5.0 (1)	5.5
44	18		5.5 (3)	3.5	5.5 (2)	5.0 (1)	5.5
45	19		(0)			(0)	

[1] Average values of density of diformazan deposition.

[2] Numbers in parenthesis indicate number of embryos.

TABLE 10

EVALUATION OF DHA- AND P-3β-HSD ACTIVITY IN ADRENAL GLANDS OF HYPOPHYSECTOMIZED EMBRYOS WHICH RECEIVED ACTH AT 24 HOUR INTERVALS

Age		Average Adrenal 3β-HSD Activity[1]							
		P-3β-HSD			DHA-3β-HSD				
Stage	Days	Non-zonated	Peripheral Zone		Central Zone	Non-zonated	Peripheral Zone		Central Zone
35	9	6.0 (2)[2]				4.7 (2)			
36	10		6.0	(2)	5.0		4.5	(2)	5.0
37	11		5.8	(3)	5.0	4.7 (2)	5.0	(1)	5.5
38	12		5.8	(4)	4.3	5.5 (1)	4.5	(3)	5.8
39	13		5.6	(4)	4.6		4.6	(4)	5.1
40	14		5.4	(5)	4.5		4.7	(3)	5.0
41	15		5.5	(3)	5.1		4.7	(3)	5.2
42	16			(0)				(0)	
43	17		5.0	(2)	4.5		5.0	(2)	6.0
44	18		6.0	(1)	5.0		6.0	(1)	5.0
45	19		5.5	(3)	4.2		5.0	(3)	5.5

[1] Average values of density of diformazan deposition.

[2] Numbers in parenthesis indicate number of embryos.

TABLE 11

EVALUATION OF DHA- AND P-3β-HSD ACTIVITY IN ADRENAL GLANDS OF HYPOPHYSECTOMIZED EMBRYOS WHICH RECEIVED HORMONE VEHICLE

Age		Average Adrenal 3β-HSD Activity[1]							
		P-3β-HSD				DHA-3β-HSD			
Stage	Days	Non-zonated	Peripheral Zone		Central Zone	Non-zonated	Peripheral Zone		Central Zone
35	9	6.0 (2)[2]	6.0	(1)	5.5	5.5 (1)	4.2	(2)	5.0
36	10		5.7	(4)	5.1	5.2 (3)	4.0	(1)	5.0
37	11		6.0	(1)	5.5	5.0 (1)			
38	12		5.7	(3)	5.0		4.5	(3)	5.3
39	13		5.5	(3)	4.8		4.5	(3)	5.3
40	14		6.0	(1)	5.0		4.5	(1)	5.0
41	15		5.2	(2)	4.5		4.5	(2)	5.2
42	16		5.0	(1)	4.5		5.0	(1)	5.5
43	17	5.5 (1)		(0)		4.5 (1)		(0)	

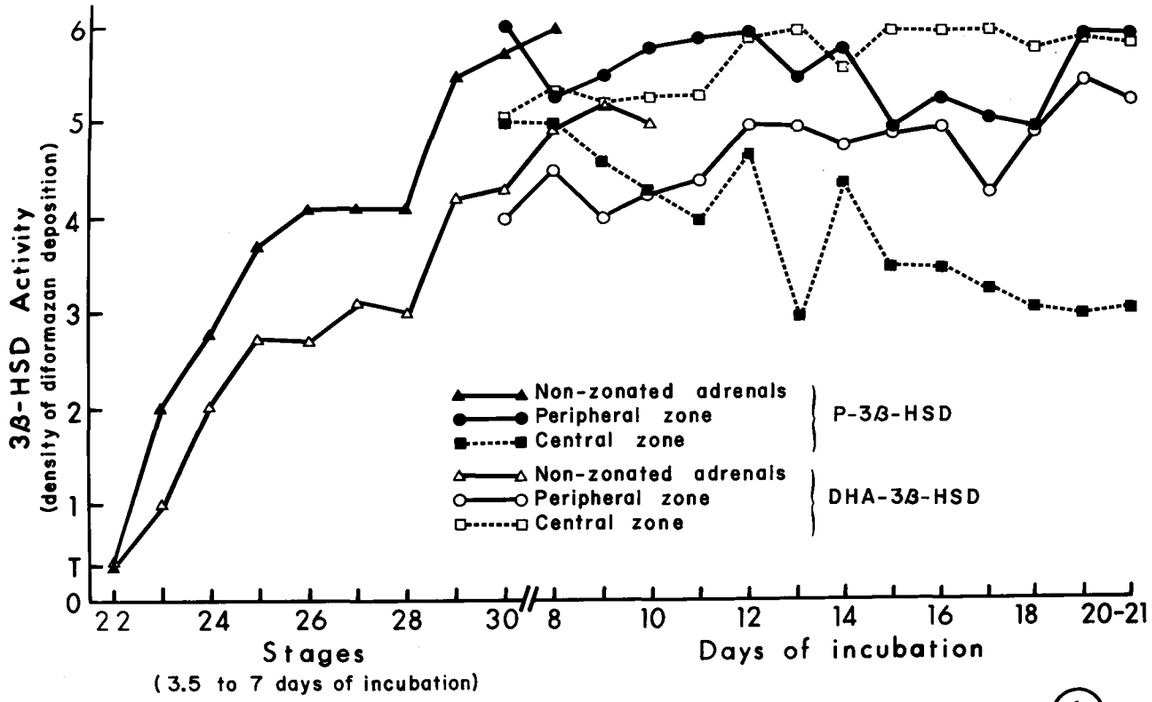
[1] Average values of density of diformazan deposition.

[2] Numbers in parenthesis indicate number of embryos.

PLATE 1

EXPLANATION OF FIGURE

1 Average adrenal 3β-HSD activity in normal embryos.

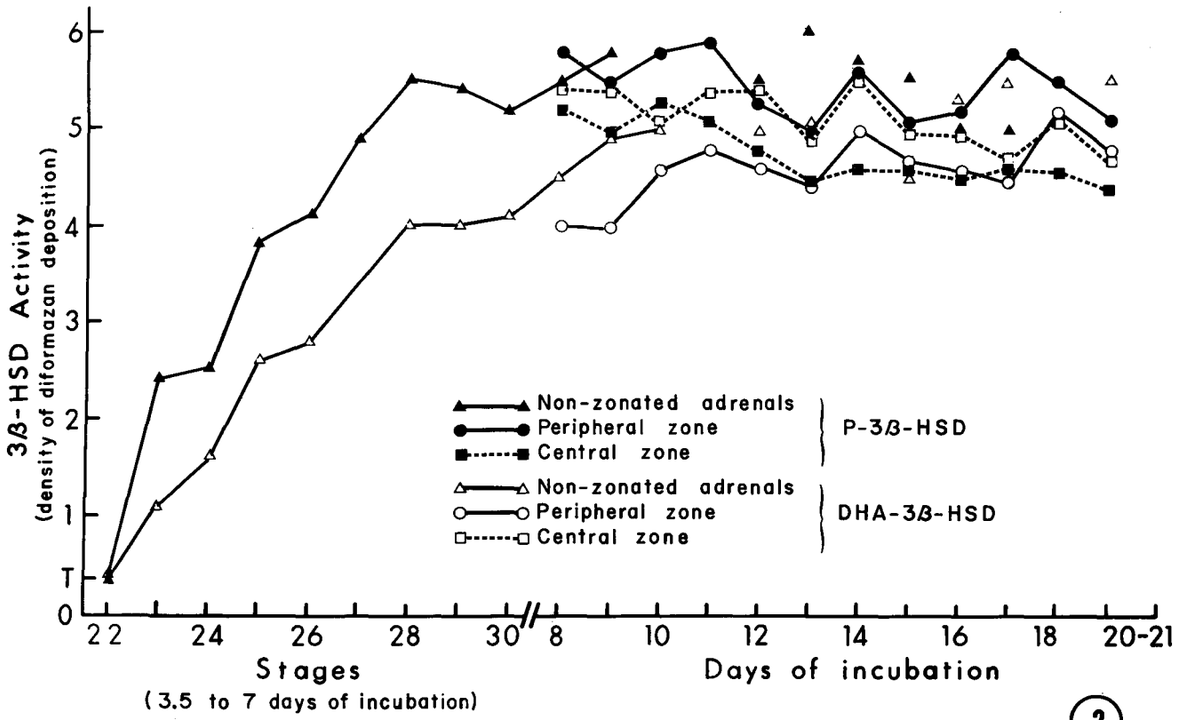


(1)

PLATE 2

EXPLANATION OF FIGURE

2 Average adrenal 3β-HSD activity in hypophysectomized embryos.

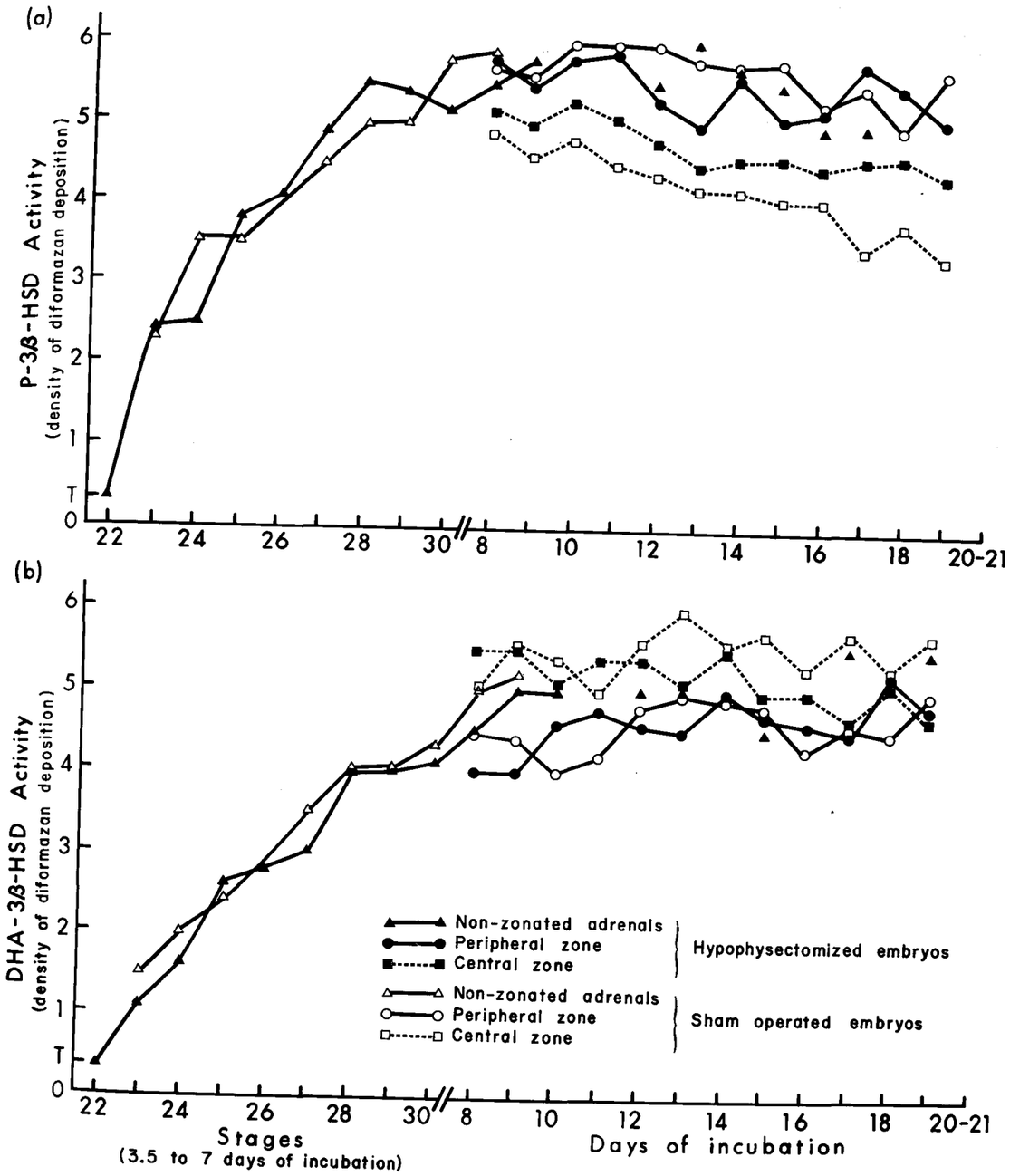


2

PLATE 3

EXPLANATION OF FIGURE

3 Average adrenal 3β-HSD activity in hypophysectomized and sham operated embryos: (a) P-3β-HSD activity, and (b) DHA-3β-HSD activity.

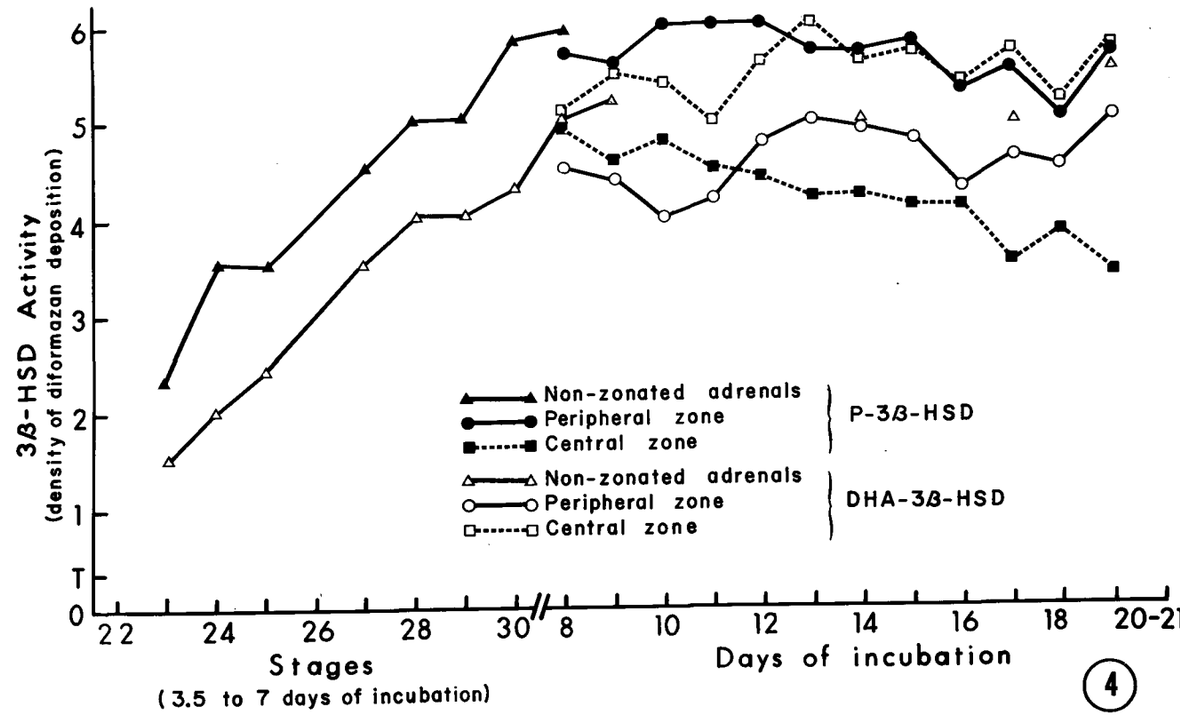


3

PLATE 4

EXPLANATION OF FIGURE

4 Average adrenal 3β-HSD activity in sham operated embryos.

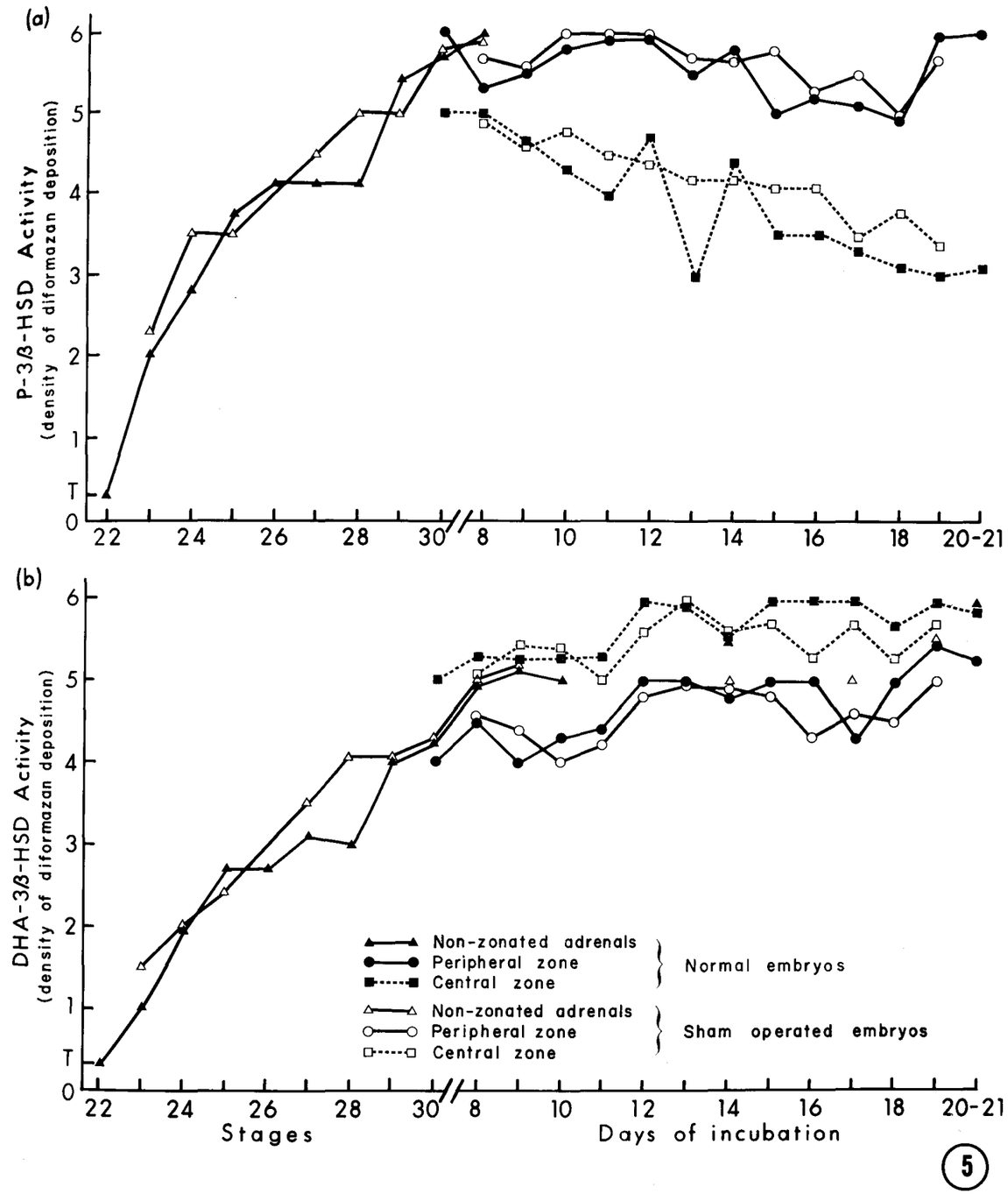


4

PLATE 5

EXPLANATION OF FIGURE

5 Average adrenal 3β-HSD activity in normal and sham operated embryos: (a) P-3β-HSD activity, and (b) DHA-3β-HSD activity.

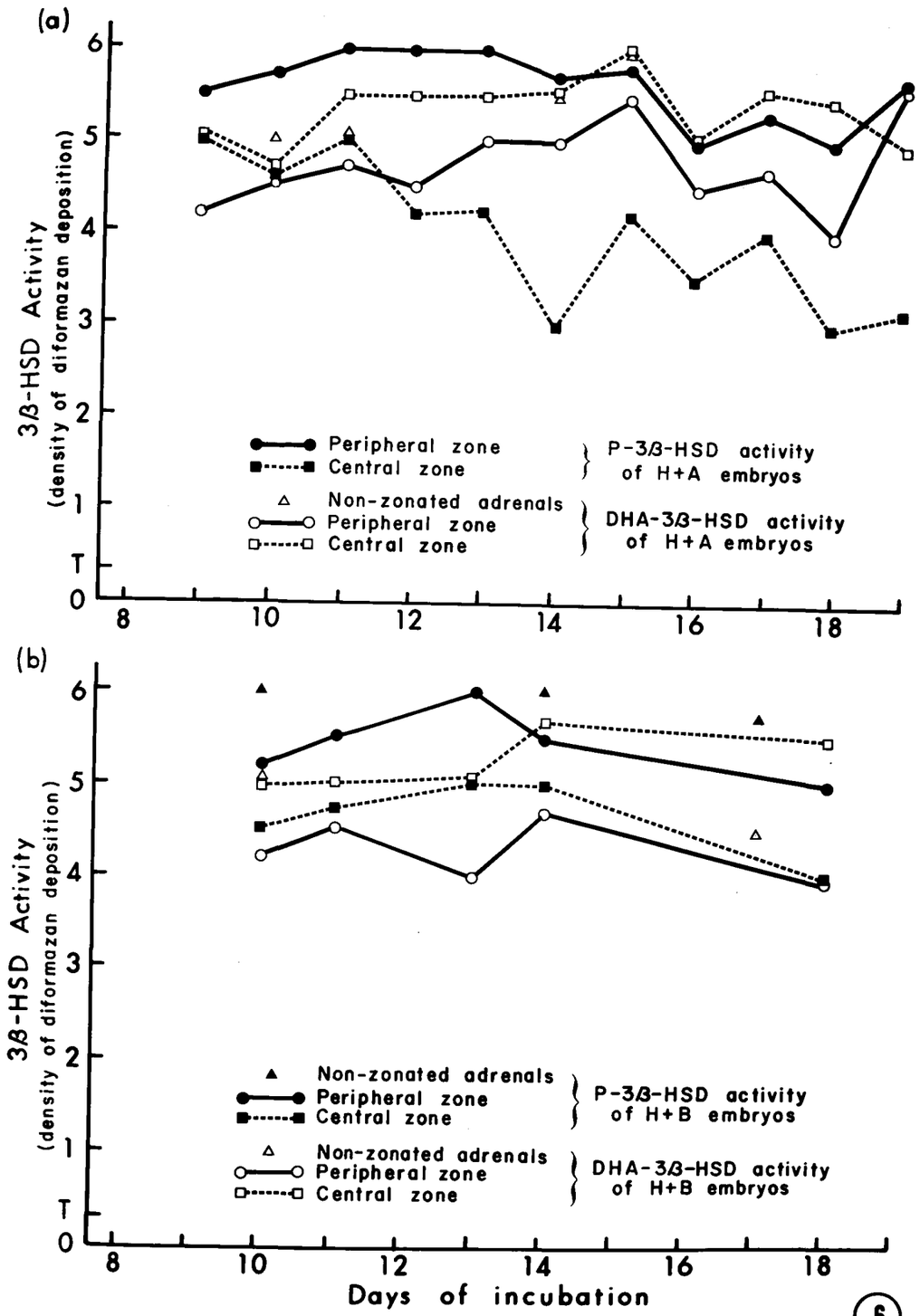


5

PLATE 6

EXPLANATION OF FIGURE

6 Average adrenal 3β-HSD activity in hypophysectomized embryos which received (a) adenohypophysis grafts (H+A embryos) and (b) brain tissue grafts (H+B embryos).

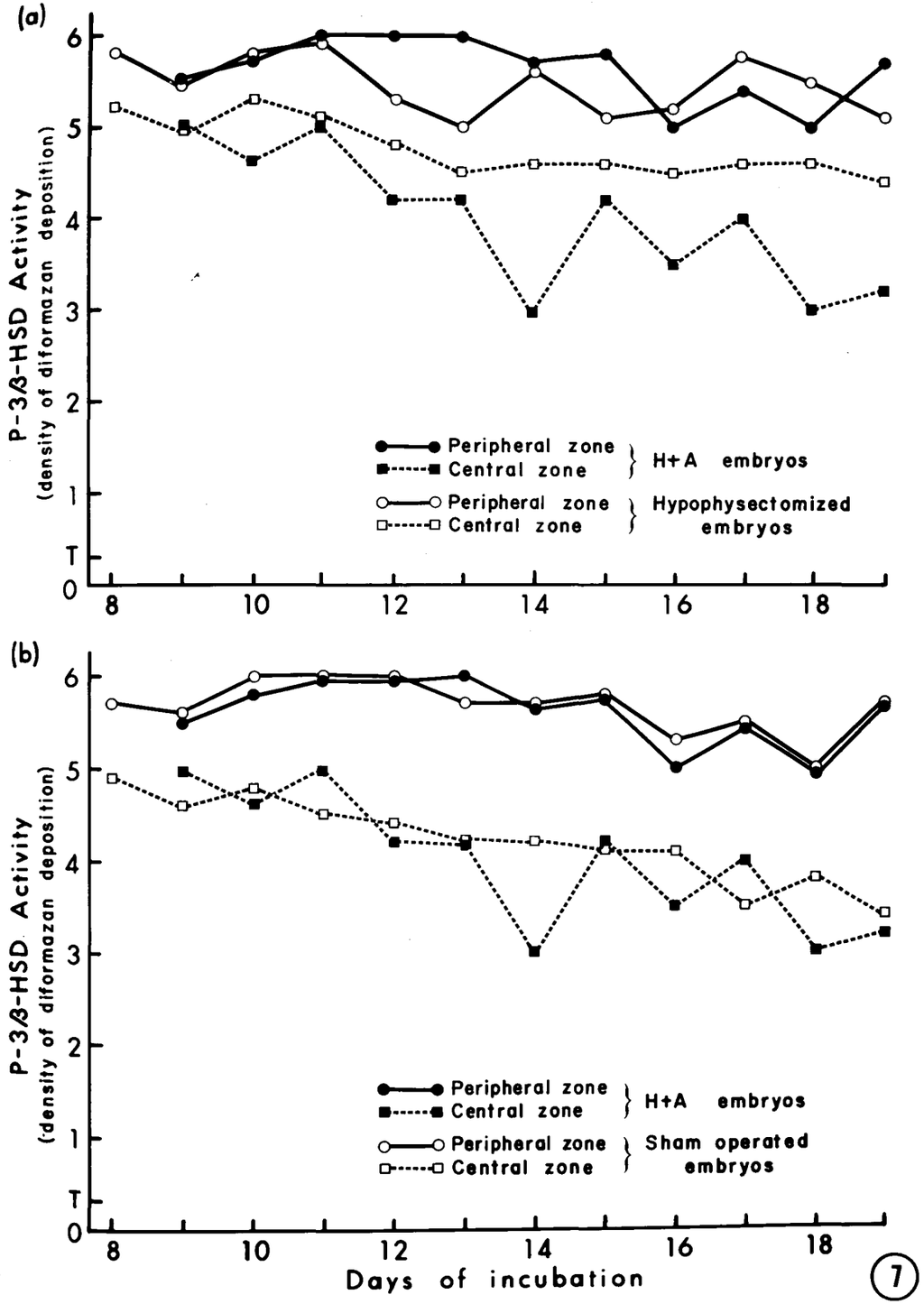


6

PLATE 7

EXPLANATION OF FIGURE

7 Average adrenal P-3β-HSD activity in hypophysectomized embryos which received adenohypophysis grafts (H+A embryos) compared with (a) non-treated hypophysectomized embryos and with (b) sham operated embryos.



7

PLATE 8

EXPLANATION OF FIGURE

8 Average adrenal DHA-3β-HSD activity in hypophysectomized embryos which received adenohypophysis grafts (H+A embryos) compared with (a) non-treated hypophysectomized embryos and with (b) sham operated embryos.

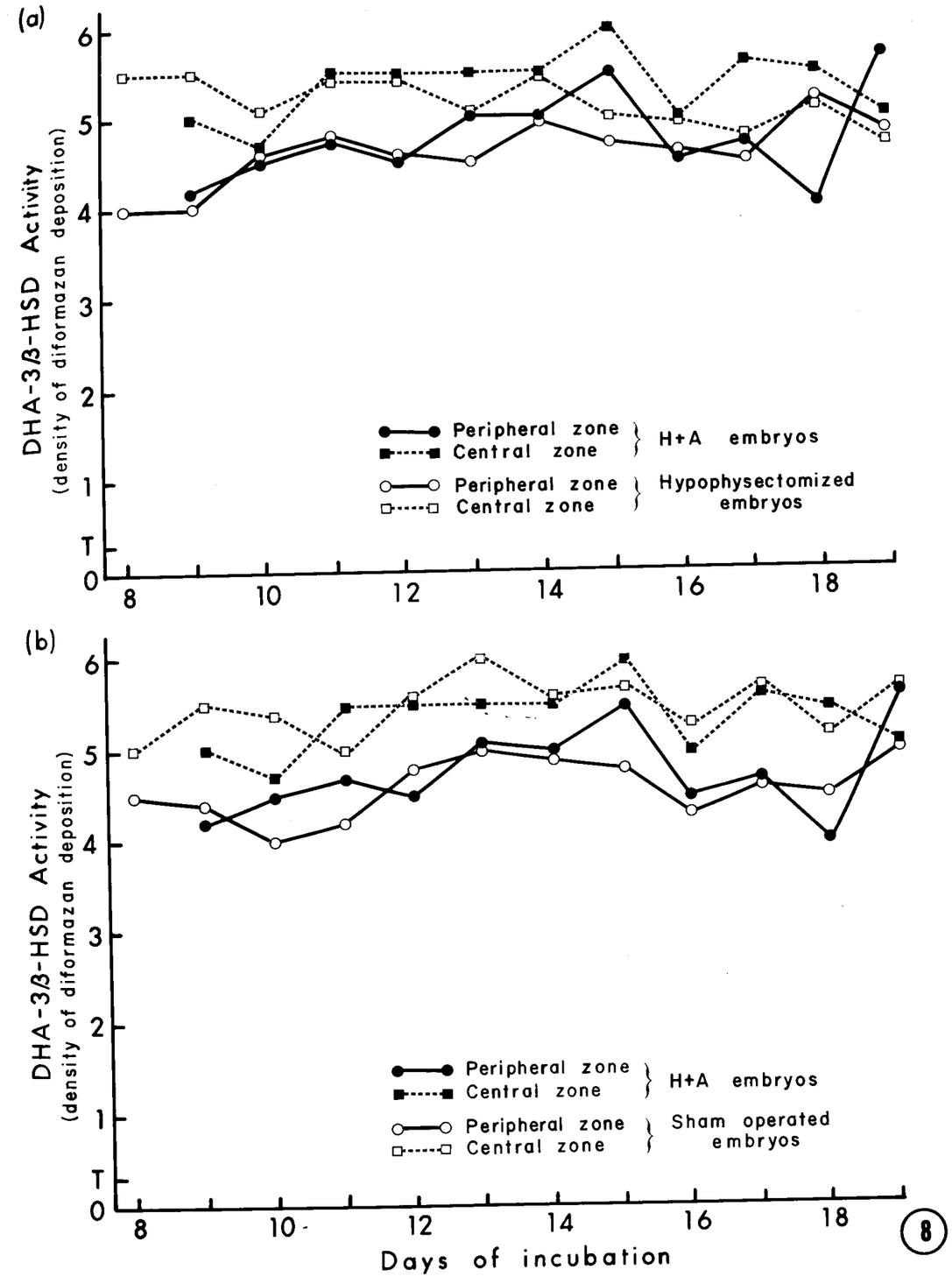
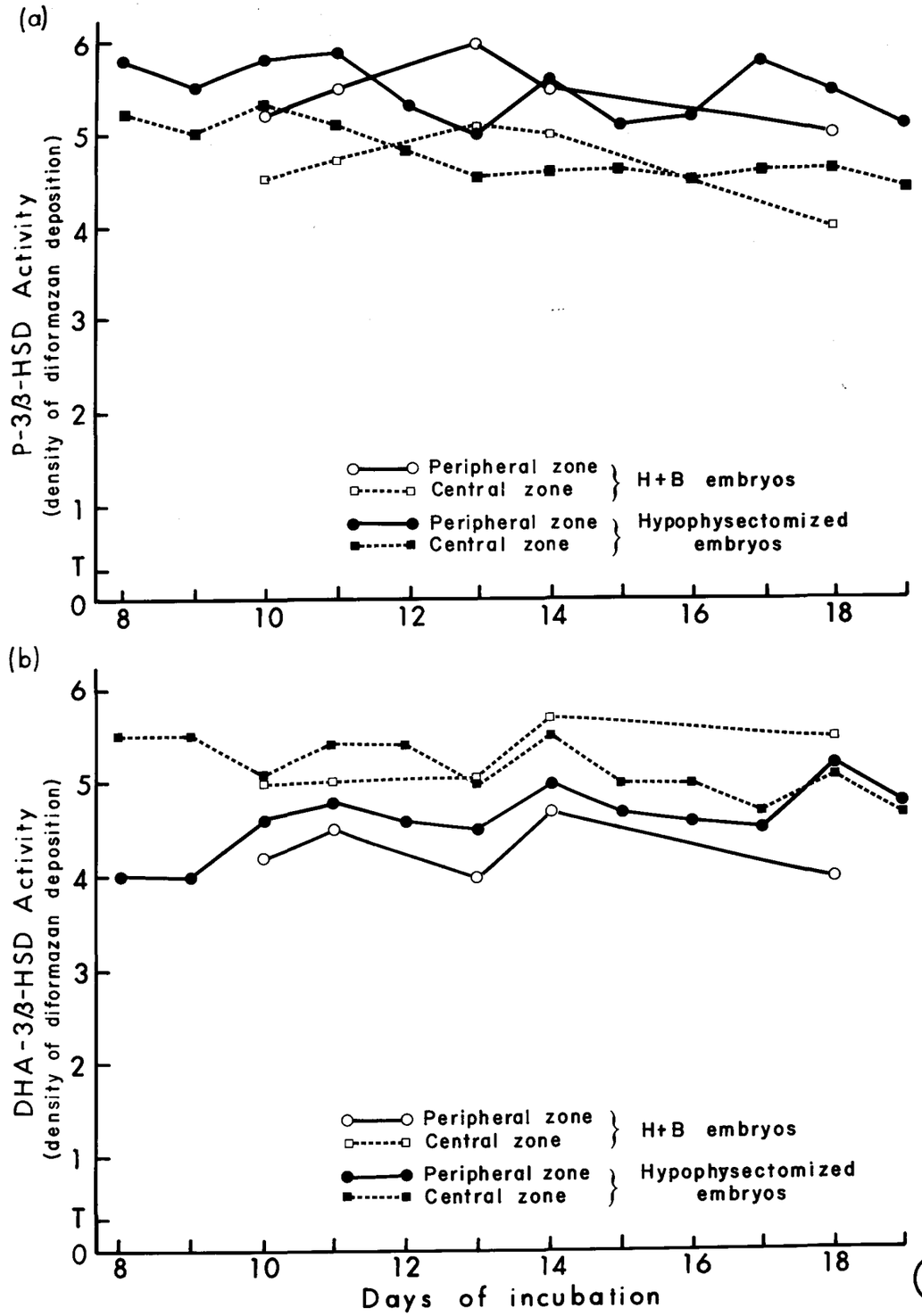


PLATE 9

EXPLANATION OF FIGURE

9 Average adrenal 3β-HSD activity in hypophysectomized embryos which received brain tissue grafts (H+B embryos) compared with non-treated hypophysectomized embryos; (a) P-3β-HSD activity, and (b) DHA-3β-HSD activity.

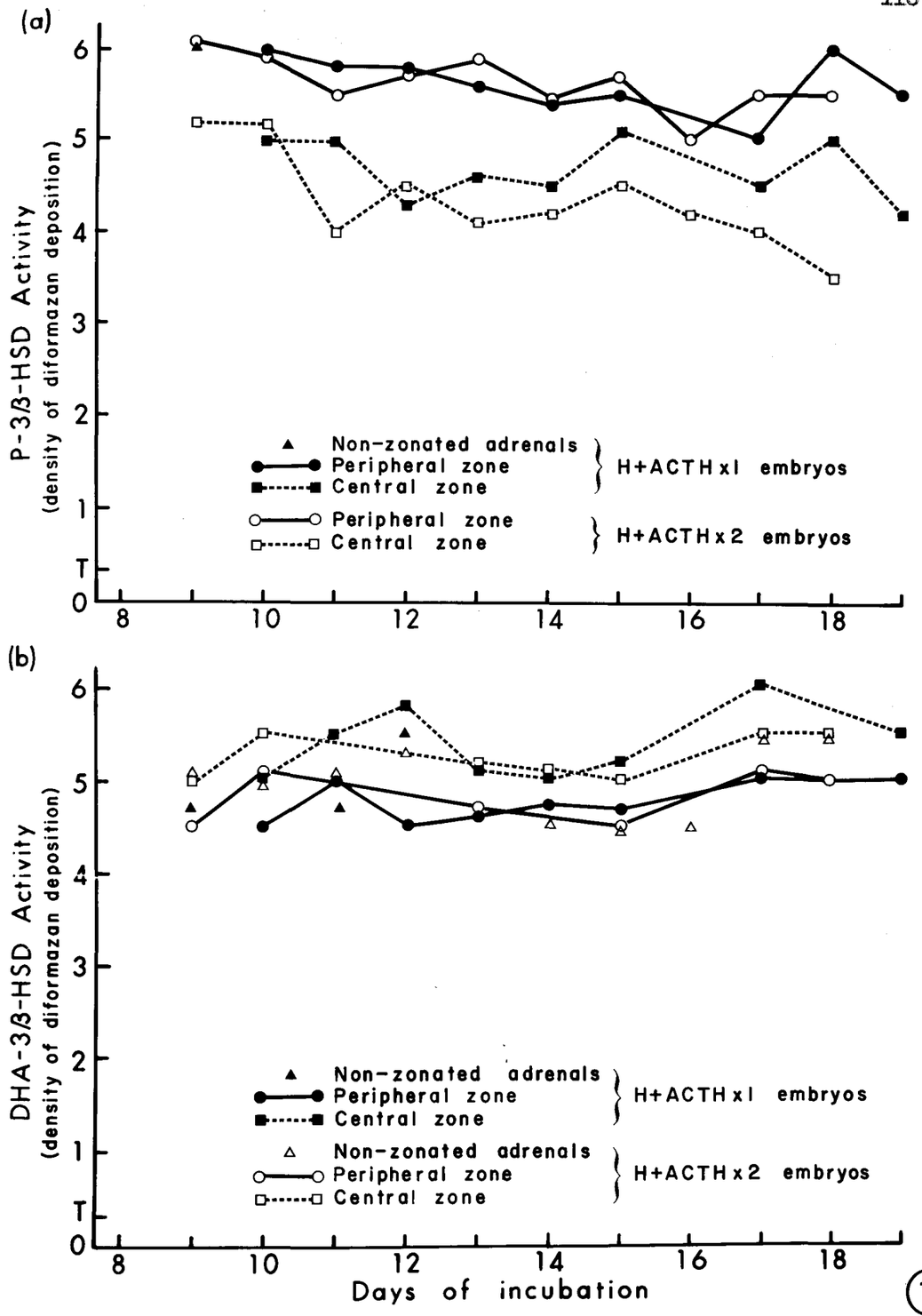


9

PLATE 10

EXPLANATION OF FIGURE

10 Average adrenal 3β-HSD activity in hypophysectomized embryos which received ACTH at 24 hour intervals (H+ACTHx1 embryos) and at 12 hour intervals (H+ACTHx2 embryos): (a) P-3β-HSD activity, and (b) DHA-3β-HSD activity.



10

PLATE 11

EXPLANATION OF FIGURE

11 Average adrenal P-3β-HSD activity in hypophysectomized embryos which received ACTH (a) at 24 hour intervals (H+ACTHx1 embryos) and (b) at 12 hour intervals (H+ACTHx2 embryos) compared with hypophysectomized embryos which received hormone vehicle (H+VEH embryos).

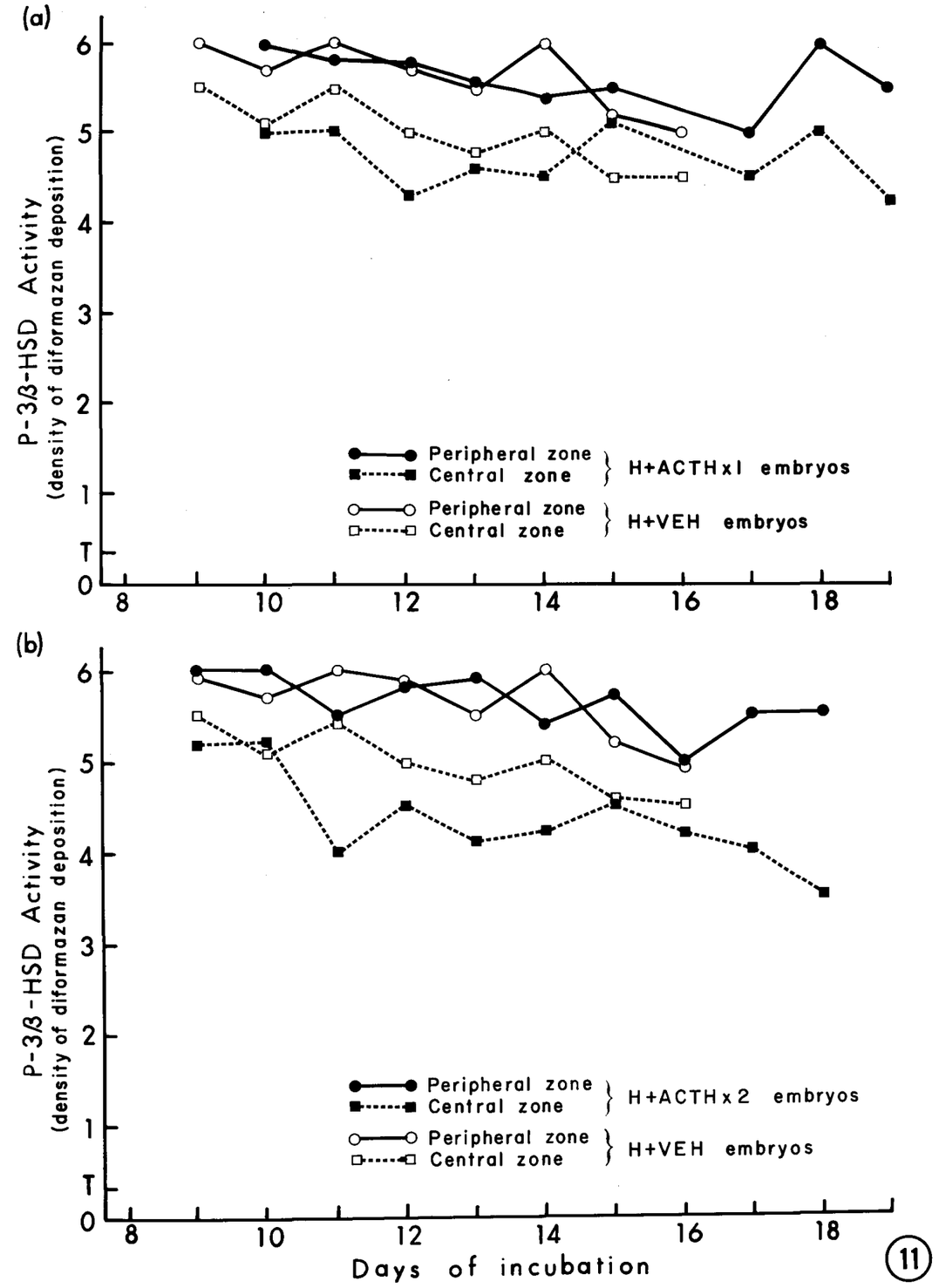


PLATE 12

EXPLANATION OF FIGURE

12 Average adrenal P-3β-HSD activity in hypophysectomized embryos which received ACTH (a) at 24 hour intervals (H+ACTHx1 embryos) and (b) at 12 hour intervals (H+ACTHx2 embryos) compared with non-treated hypophysectomized embryos.

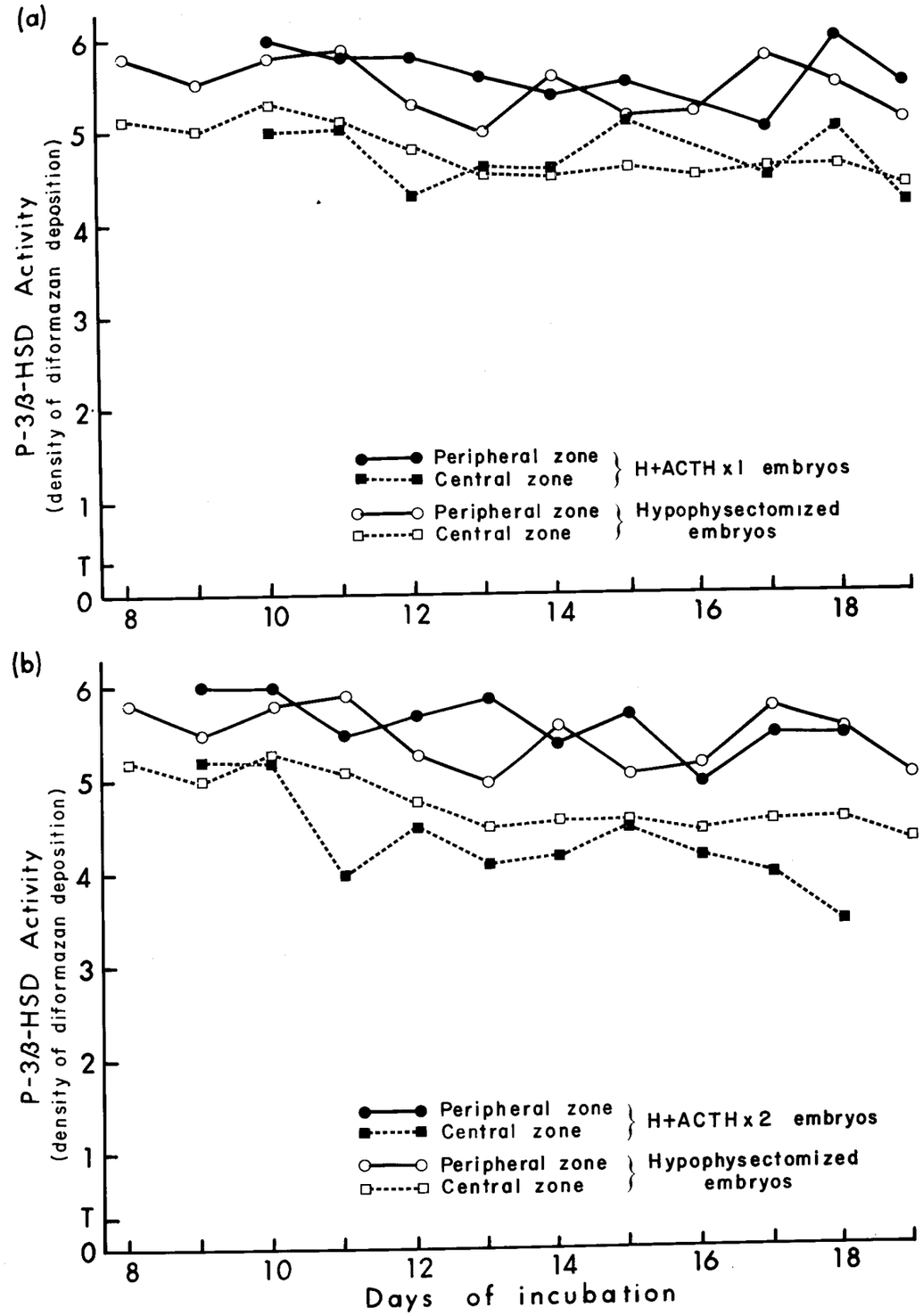
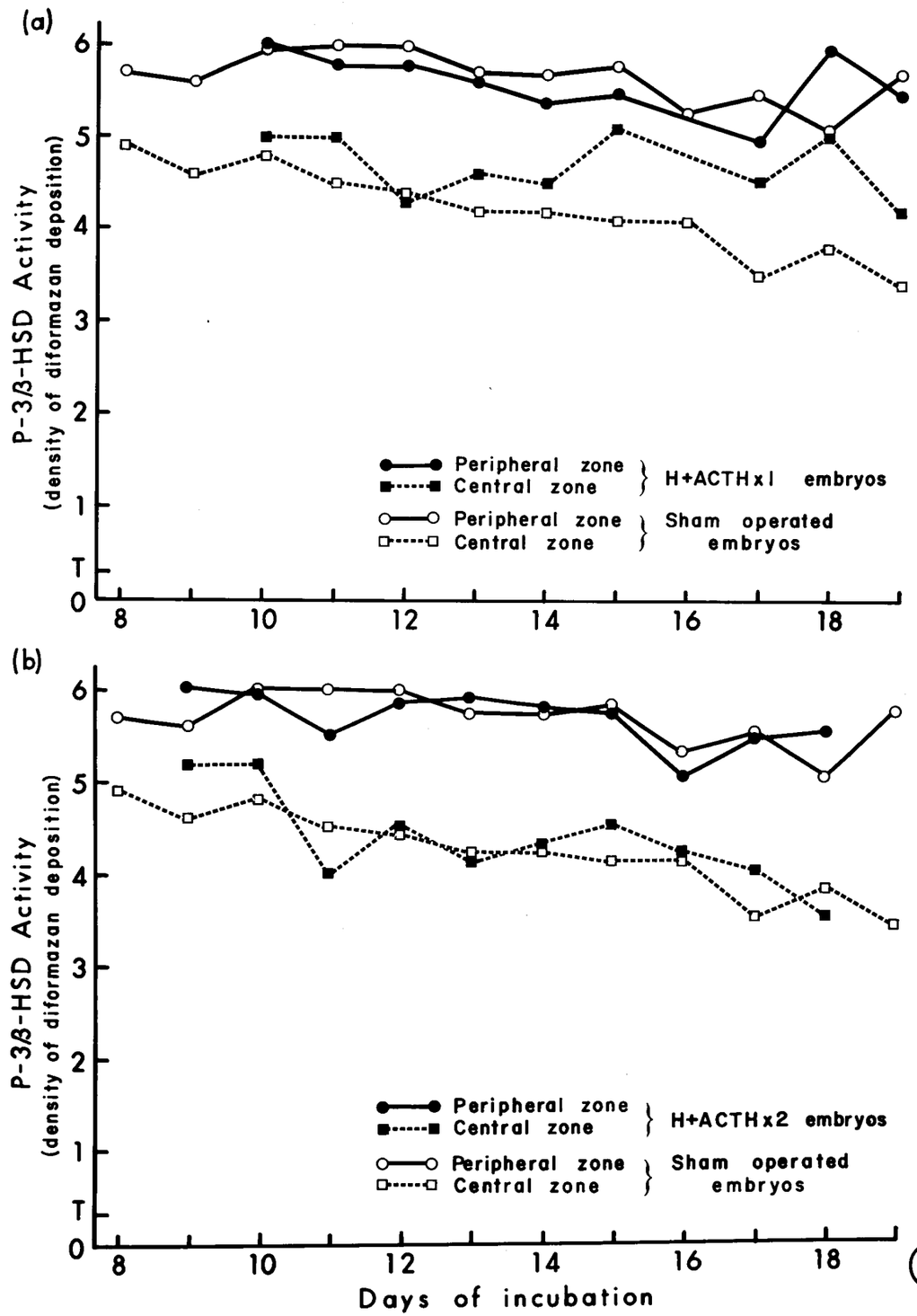


PLATE 13

EXPLANATION OF FIGURE

13 Average adrenal P-3β-HSD activity in hypophysectomized embryos which received ACTH (a) at 24 hour intervals (H+ACTHx1 embryos) and (b) at 12 hour intervals (H+ACTHx2 embryos) compared with sham operated embryos.

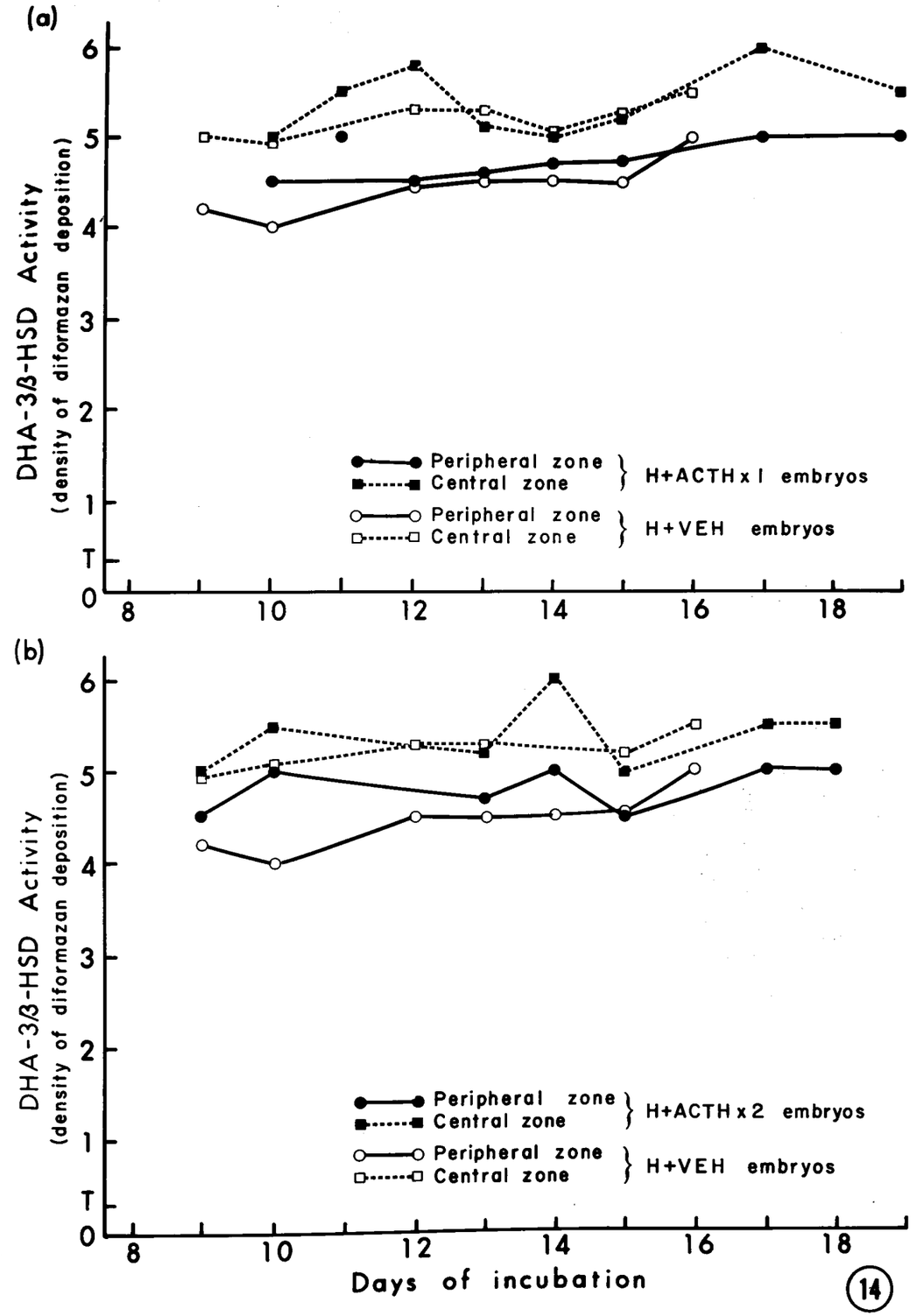


13

PLATE 14

EXPLANATION OF FIGURE

14 Average adrenal DHA-3β-HSD activity of hypophysectomized embryos which received ACTH (a) at 24 hour intervals (H+ACTHx1 embryos) and (b) at 12 hour intervals (H+ACTHx2 embryos) compared with hypophysectomized embryos which received hormone vehicle (H+VEH embryos).



14

PLATE 15

EXPLANATION OF FIGURE

15 Average adrenal DHA-3β-HSD activity of hypophysectomized embryos which received ACTH (a) at 24 hour intervals (H+ACTHx1 embryos) and (b) at 12 hour intervals (H+ACTHx2 embryos) compared with non-treated hypophysectomized embryos.

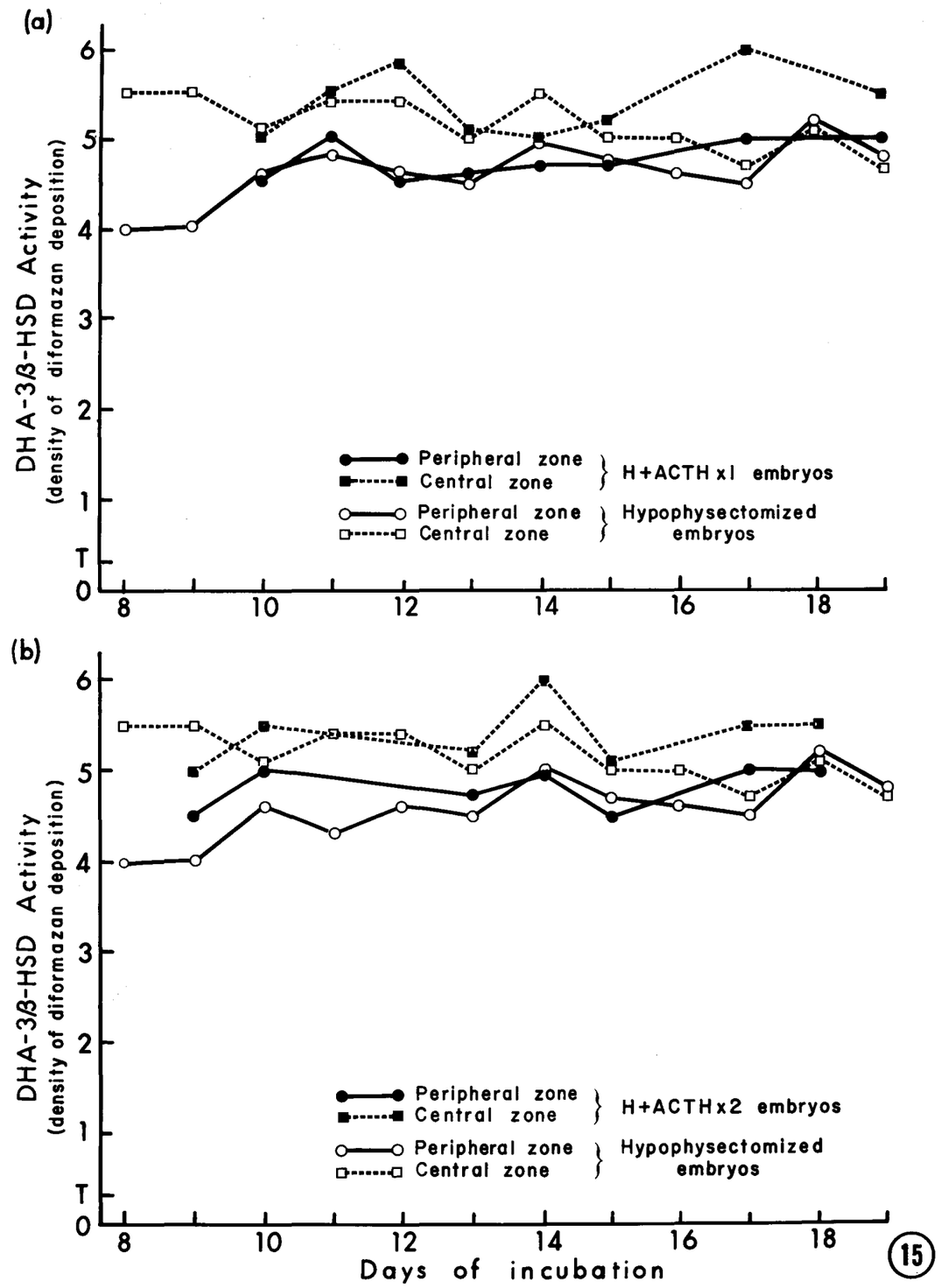


PLATE 16

EXPLANATION OF FIGURE

16 Average adrenal DHA-3β-HSD activity of hypophysectomized embryos which received ACTH (a) at 24 hour intervals (H+ACTHx1 embryos) and (b) at 12 hour intervals (H+ACTHx2 embryos) compared with sham operated embryos.

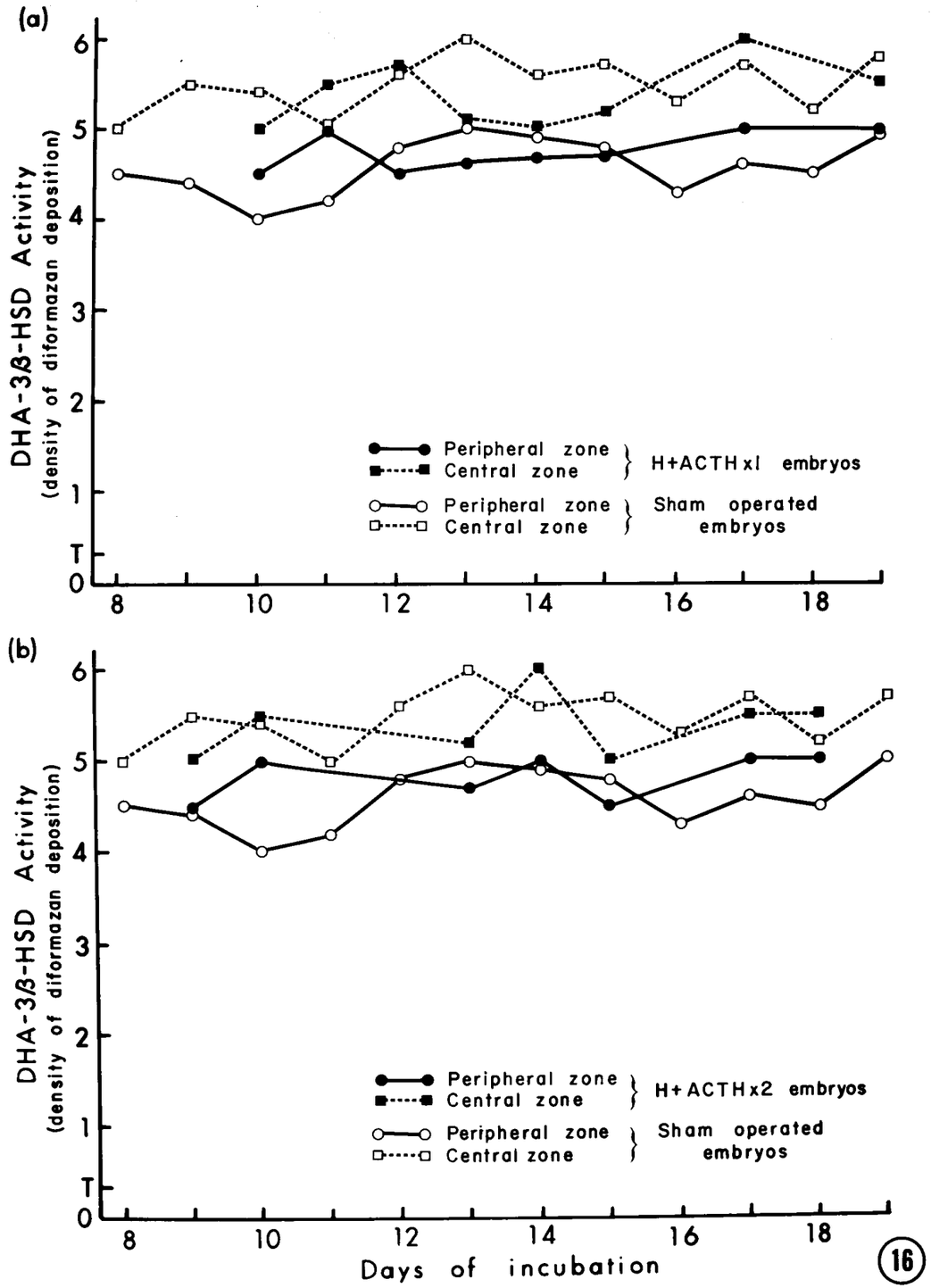
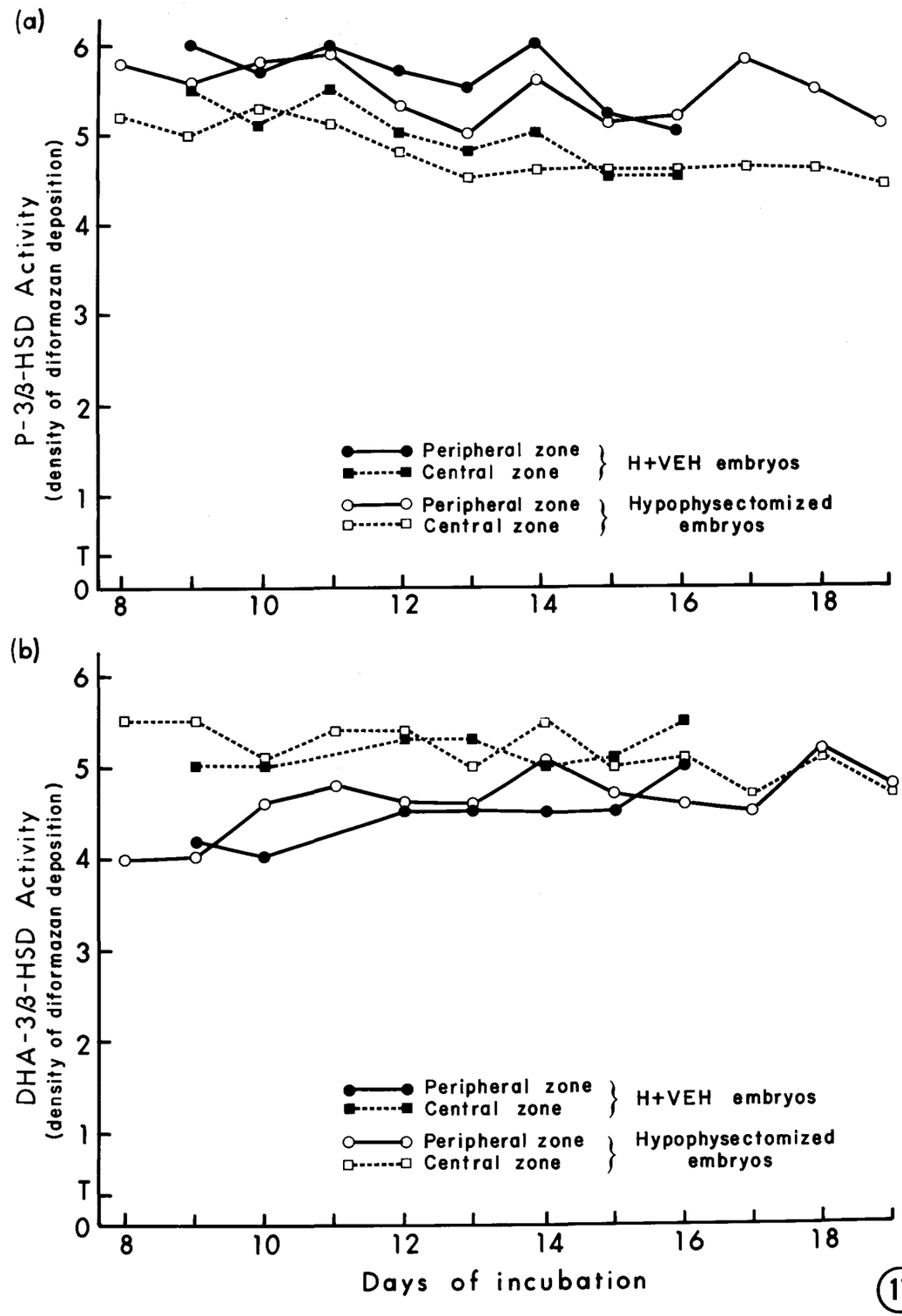


PLATE 17

EXPLANATION OF FIGURE

17 Average adrenal 3β-HSD activity of hypophysectomized embryos which received hormone vehicle (H+VEH embryos) compared with non-treated hypophysectomized embryos: (a) P-3β-HSD activity, and (b) DHA-3β-HSD activity.



17

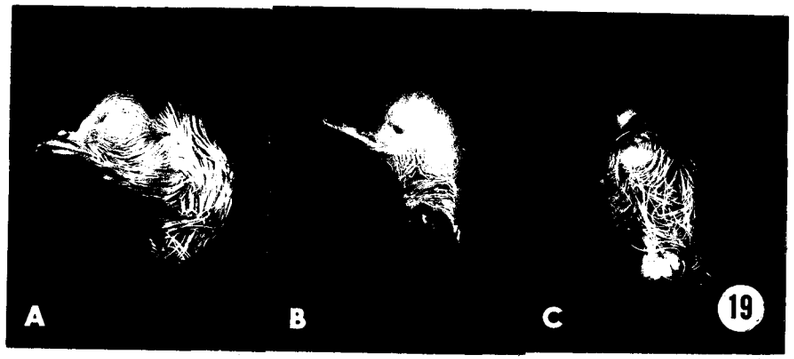
PLATE 18

EXPLANATION OF FIGURES

18 Whole mount of a normal stage 10 (33 - 38 hours) chick embryo. Hypophysectomy by partial decapitation was accomplished by making a transverse cut at the mid-region of the mesencephalon between the two lines and removing all tissue anterior of this level. X26.

19 Heads of 19 day white Leghorn embryos: A, normal embryo. B and C, embryos hypophysectomized by partial decapitation. Both hypophysectomized embryos lack eyes, comb, nostrils and upper beak. Embryo C also lacks lower beak. X1.

20 Section of an adrenal of a stage 29 (6 - 6.5 days) normal embryo processed for histochemically demonstrable 3 -HSD using the substrate DHA. The nuclei are stained with carmine and fine dark blue diformazan granules representing an activity of 4 can be seen. X450.



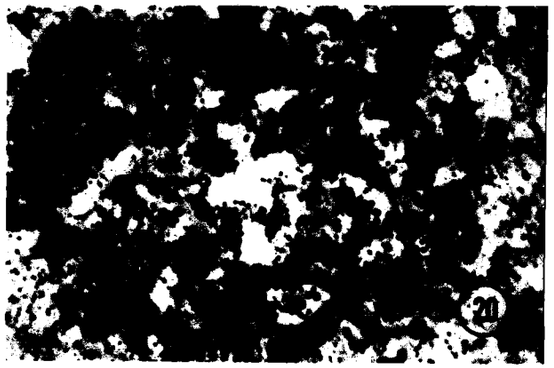


PLATE 19

EXPLANATION OF FIGURES

21 Photomicrographs representing the divisions of the arbitrary scale employed in evaluating the intensity of enzyme activity. The number on each photomicrograph represents the estimated activity. X450.

22 Cross section of a normal stage 22 (3.5 - 4 day) embryo at the level of the origin of the omphalomesenteric artery (O) showing the location of the thickened peritoneal epithelium (PE), primordial adrenocortical cells (AC), mesonephros (M), aorta (A) and subcardinal vein (SV). X100.

23 A higher magnification of the normal stage 22 (3.5 - 4 day) embryo shown in figure 22. Primordial adrenocortical cells (AC) are shown migrating from the perit neal epithelium (PE) to a point in the mesenchyme dorsal to the subcardinal vein (SV) and between the aorta (A) and mesonephros (M). X250.

24 Trace amounts of P-3β-HSD activity in the thickened peritoneal epithelium (PE) and in the migrating adrenocortical cells (AC) of a normal stage 22 (3.5 - 4 day) embryo. Compare figures 25 and 26. X450.

25 Trace amounts of DHA-3β-HSD activity in the thickened peritoneal epithelium (PE) and in the migrating adrenocortical cells (AC) of the stage 22 embryo shown in figure 24. Compare figure 26. X450.

26 Control section of the embryo shown in figures 24 and 25 incubated in media which lacked the substrate. No diformazan granules are present. X450.

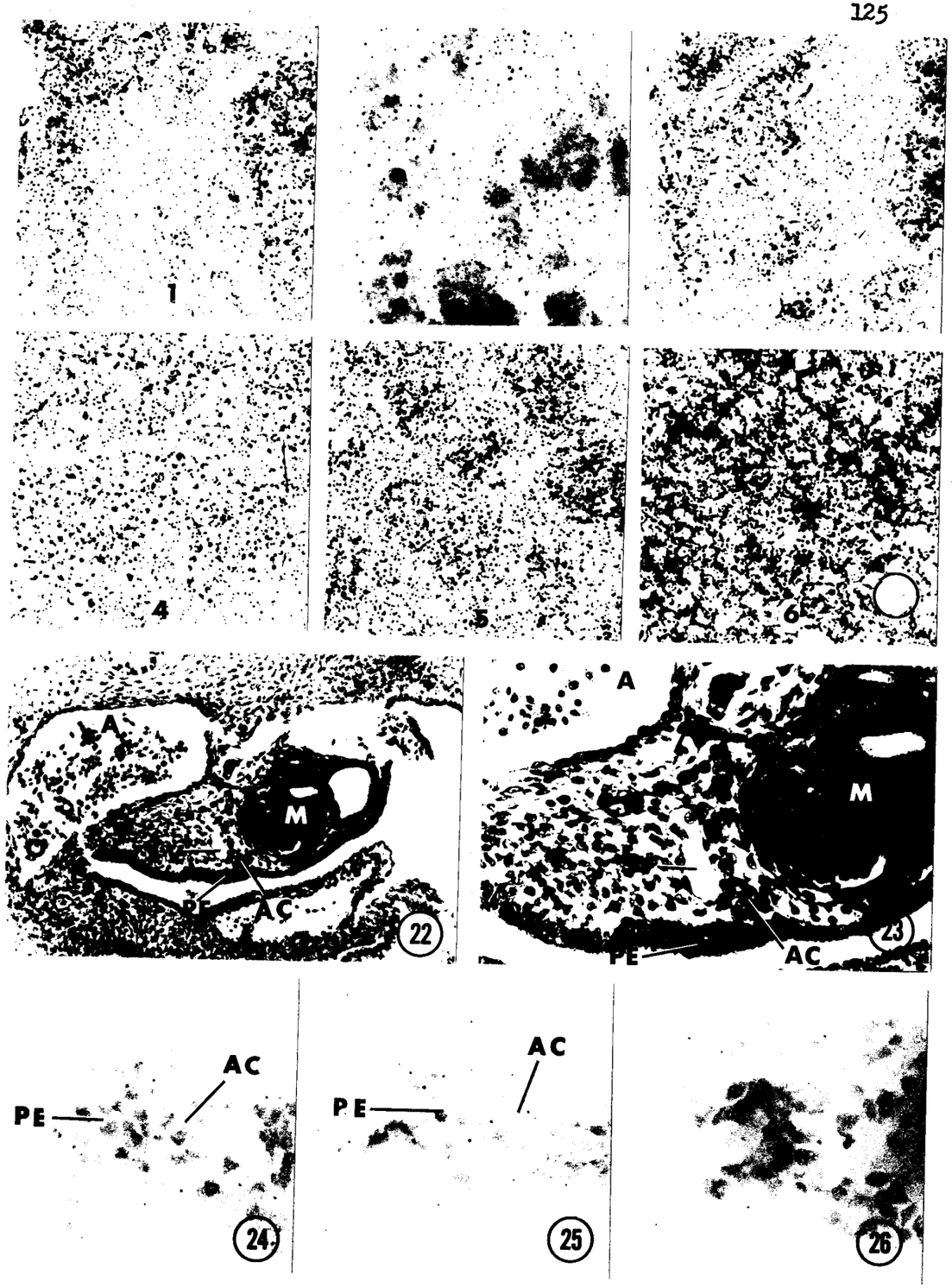


PLATE 20

EXPLANATION OF FIGURES

27 Cross section of a normal stage 23 (4 day) embryo showing a chain of migrating adrenocortical cells (AC') lateral to the subcardinal vein (SV) and extending from the peritoneal epithelium (PE) to a point in the mesenchyme between the mesonephros (M) and aorta (A) where they form scattered groups of adrenocortical cells (AC"). Migrating chromaffin cells (CC) are also shown. X100.

28 A higher magnification of the stage 23 (4 day) embryo shown in figure 27 showing the chain of migrating adrenocortical cells (AC') and the scattered groups of two or three adrenocortical cells (AC"). The thickened peritoneal epithelium (PE), mesonephros (M), subcardinal vein (SV) and migrating chromaffin cells (CC) are also shown. X250.

29 P-3β-HSD activity of 2 intensity in the adrenocortical cells of a normal stage 23 (4 day) embryo. Compare figures 30 and 31. X450.

30 DHA-3β-HSD activity of 1 intensity in the adrenocortical cells of the embryo shown in figure 29. Compare figure 31. X450.

31 Control section of the adrenocortical cells of the embryo shown in figures 29 and 30 incubated in media which lacked the substrate. No diformazan granules are present. X450.

32 P-3β-HSD activity of 4 intensity in the adrenocortical cells of a normal stage 26 (5 day) embryo. Compare figures 33 and 34. X450.

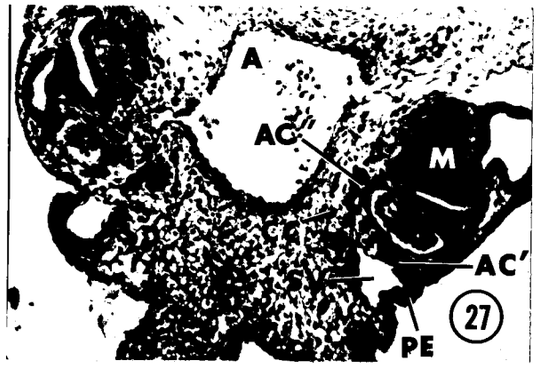
33 DHA-3β-HSD activity of 2 intensity in the adrenocortical cells of the embryo shown in figure 32. Compare figure 34. X450.

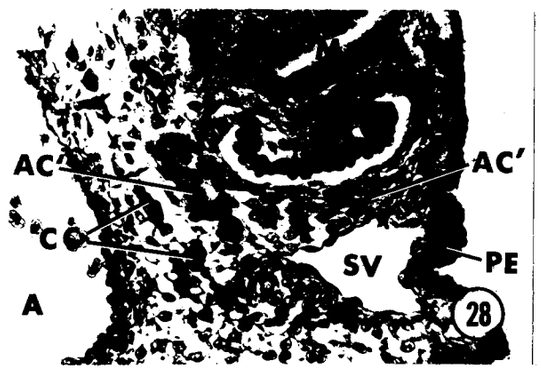
34 Control section of the adrenocortical cells of the embryo shown in figures 32 and 33 incubated in media which lacked the substrate. Trace amounts of non-specific diformazan are present. X450.

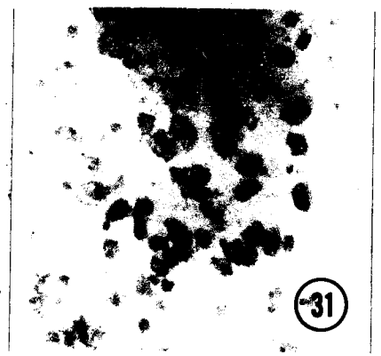
35 P-3β-HSD activity of 6 intensity in the adrenocortical cells of a normal 8 day embryo. Compare figures 36 and 37. X450.

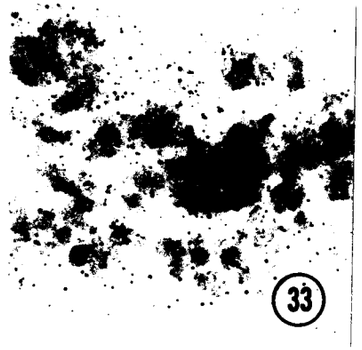
36 DHA-3β-HSD activity of 5 intensity in the adrenocortical cells of the embryo shown in figure 35. Compare figure 37. X450.

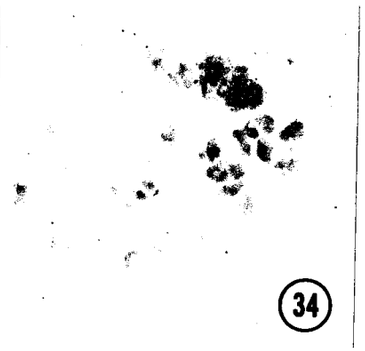
37 Control section of the adrenocortical cells of the embryo shown in figures 35 and 36 incubated in media which lacked the substrate. Trace amounts of non-specific diformazan are present. X450.

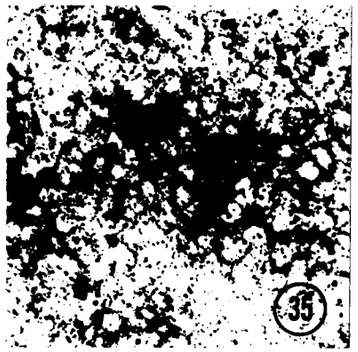


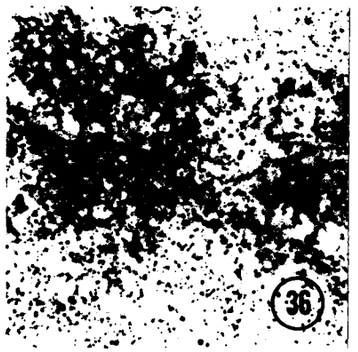












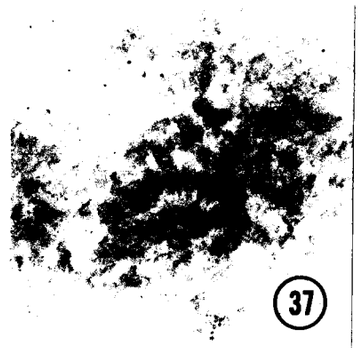


PLATE 21

ELPLANATION OF FIGURES

38 P-3β-HSD activity in a section of adrenal from a normal 8 day embryo showing the beginning of zone formation. The activity at the periphery is higher than in the central portion of the gland. Compare figure 41. X112.

39 Higher magnification of the P-3β-HSD activity shown in figure 38. The peripheral zone (P) activity is 6 and the central zone (C) activity is 5. Compare figures 40 and 42. X450.

40 Control section of the adrenal of the embryo shown in figures 39 and 42 incubated in media which lacked the substrate. No diformazan granules are present in the peripheral zone (P) or in the central zone (C). X450.

41 DHA-3β-HSD activity in a section of adrenal from the embryo shown in figure 38 showing the beginning of zone formation. The activity at the periphery is lower than in the central portion of the gland. See figure 42. X112.

42 Higher magnification of the DHA-3β-HSD activity shown in figure 41. The peripheral zone (P) activity is 3 and the central zone (C) activity is 5. Compare figures 39 and 40. X450.

43 P-3β-HSD activity in a section of adrenal from a normal nine day embryo. The diameter of the central zone of low activity is about equal to the width of the peripheral zone of higher activity. Compare figure 46. X112.

44 Higher magnification of the P-3β-HSD activity shown in figure 43. Peripheral zone (P) activity is 6 and central zone (C) activity is 4. Compare figures 45 and 47. X450.

45 Control section of the adrenal of the embryo shown in figures 44 and 47 incubated in media which lacked the substrate. Trace amounts of non-specific diformazan are present in the peripheral zone (P) and in the central zone (C). X450.

46 DHA-3β-HSD activity in a section of adrenal from the normal nine day embryo shown in figure 43. See figure 47. X450.

47 Higher magnification of the DHA-3β-HSD activity in figure 46. Peripheral zone (P) activity is 3 and central zone (C) activity is 5. Compare figures 44 and 45. X450.

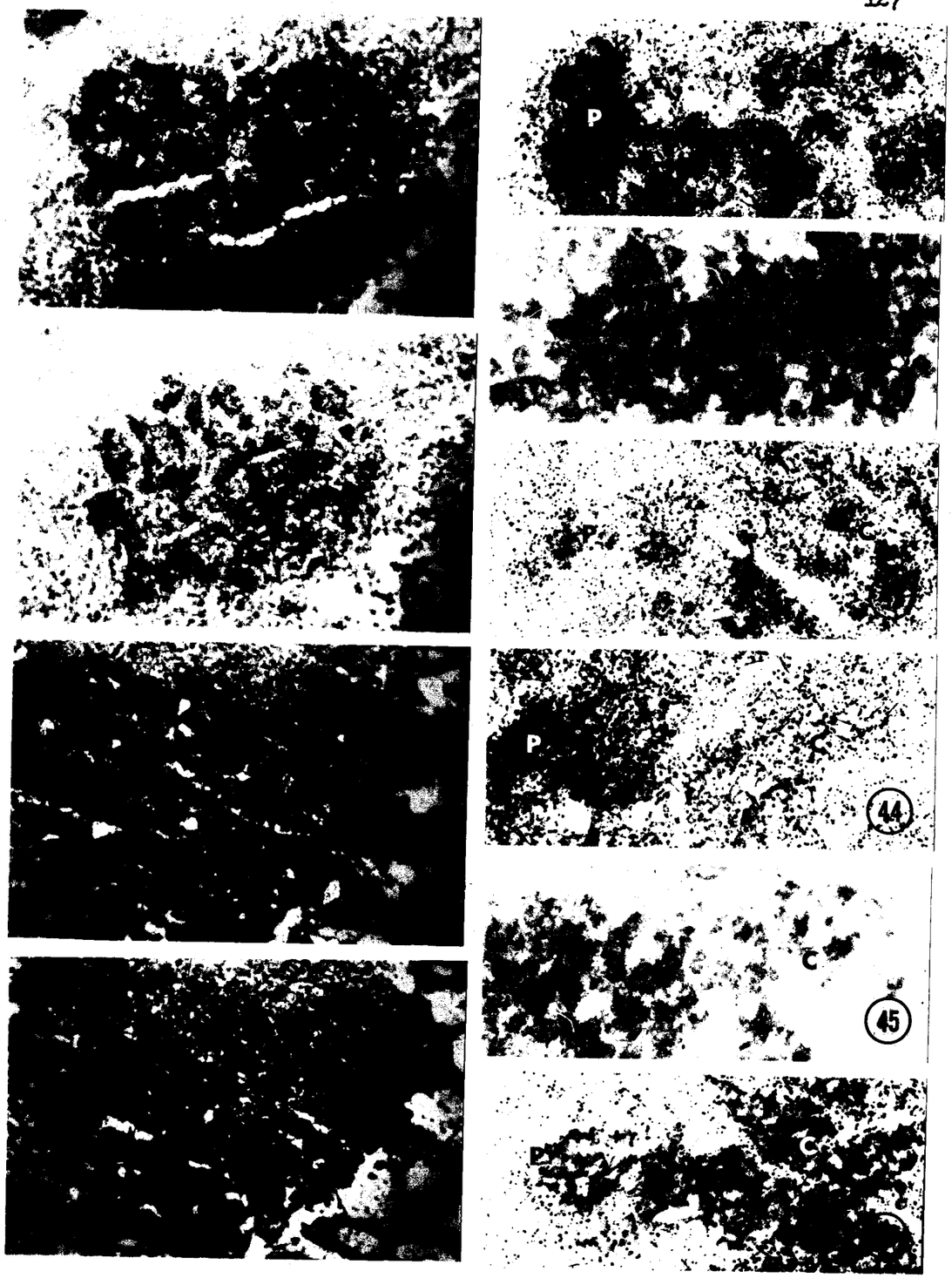


PLATE 22

EXPLANATION OF FIGURES

48 P-3β-HSD activity in a section of adrenal from a normal 11 day embryo. The width of the peripheral zone of the peripheral zone of high activity is about half of the width of the central zone of lower activity but wider than the peripheral zone in the adrenal of the 15 day embryo shown in figure 54. Compare figure 51. X56.

49 Higher magnification of the peripheral zone P-3β-HSD activity of 5 intensity shown in figure 48. Compare figure 50. X450.

50 Higher magnification of the central zone P-3β-HSD activity of 4 intensity shown in figure 48. Compare figure 49. X450.

51 DHA-3β-HSD activity in a section of adrenal from the normal 11 day embryo shown in figure 48. The width of the peripheral zone of low activity is about half of the width of the central zone of higher actvity but wider than the peripheral zone in the adrenal of the 15 day embryo shown in figure 57. X56.

52 Higher magnification of the peripheral zone DHA-3β-HSD activity of 4 intensity shown in figure 51. Compare figure 53. X450.

53 Higher magnification of the central zone DHA-3β-HSD activity of 5 intensity shown in figure 51. X450.

54 P-3β-HSD activity in a section of adrenal from a normal 15 day embryo showing a narrow peripheral zone of high activity and a wide central zone of lower activity. Compare figure 57. X56.

55 Higher magnification of the peripheral zone P-3β-HSD activity of 4 intensity in figure 54. Compare figure 56. X450.

56 Higher magnification of the central zone P-3β-HSD activity of 3 intensity in figure 54. Compare figure 55. X450.

57 DHA-3β-HSD activity in a section of adrenal from the normal 15 day embryo shown in fugure 54 showing a narrow peripheral zone of low activity and a wide central zone of high activity. X450.

58 Higher magnification of the peripheral zone DHA-3β-HSD activity of 4 intensity shown in figure 57. Compare figure 59. X450.

59 Higher magnification of the central zone DHA-3β-HSD activity of 5 intensity shown in figure 57. Compare figure 58. X450.

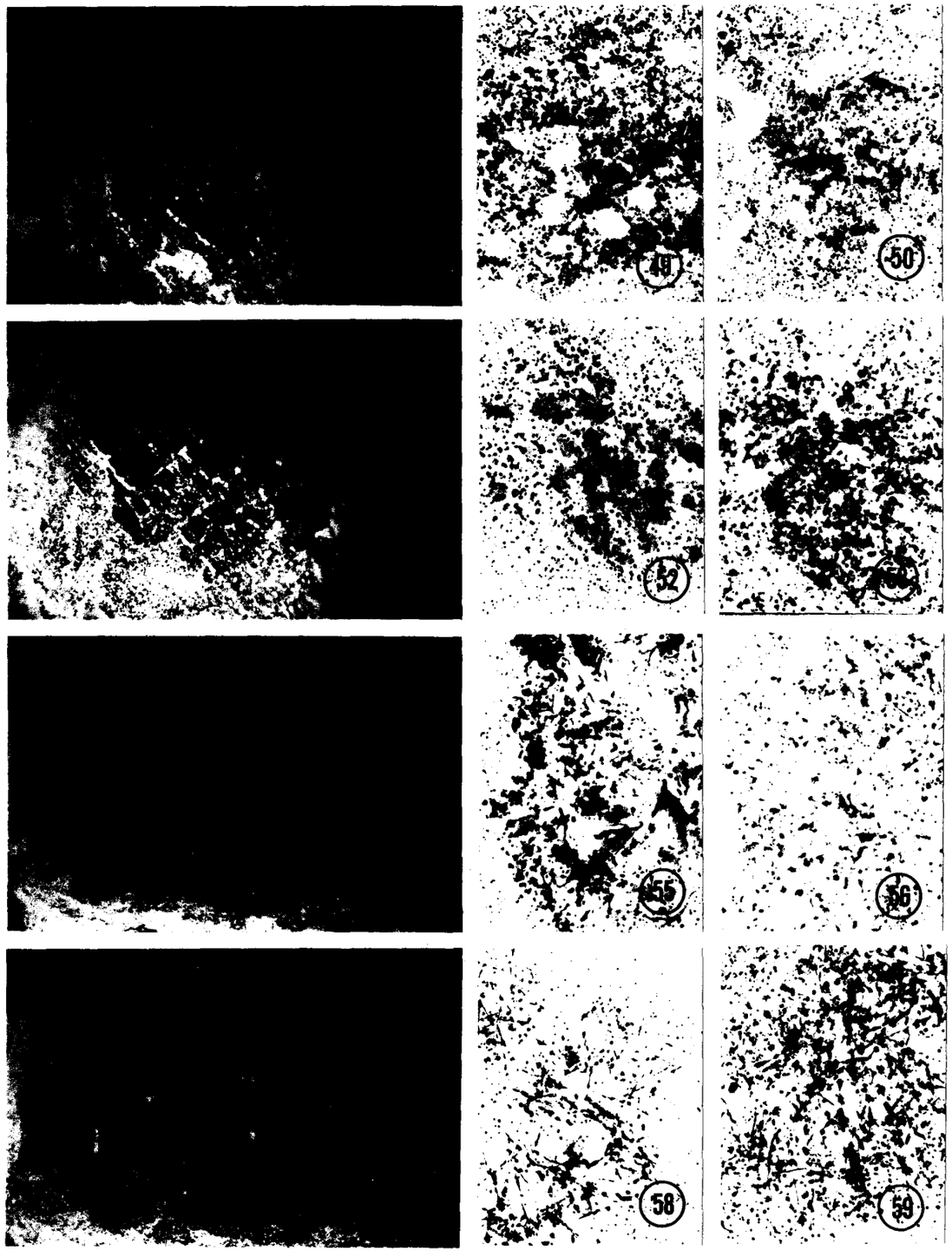


PLATE 23

EXPLANATION OF FIGURES

60 P-3β-HSD activity in a section of adrenal from a normal 20 day embryo which was in the process of hatching showing a narrow peripheral zone of high activity and a wide central zone of low activity. Compare figure 63. X40.

61 Higher magnification of the peripheral zone P-3β-HSD activity of 5 intensity shown in figure 60. Compare figures 62 and 67. X450.

62 Higher magnification of the central zone P-3β-HSD activity of 3 intensity shown in figure 60. Compare figures 61 and 67. Central zone activity is lower than that of the 11 day embryo shown in figure 50. X450.

63 DHA-3β-HSD activity in a section of adrenal from the normal 20 day embryo shown in figure 60 showing a narrow peripheral zone of low activity and a wide central zone of high activity. X40.

64 Higher magnification of the peripheral zone DHA-3β-HSD activity of 5 intensity shown in figure 63. Compare figures 65 and 67. X450.

65 Higher magnification of the central zone DHA-3β-HSD activity of 6 intensity shown in figure 63. Compare figures 64 and 67. X450.

66 Section of the adrenal from the normal 20 day embryo shown in figure 60. Diformazan granules indicating P-3β-HSD activity can be seen in the cortical cord (C) but are not present in the center of the large accumulation of chromaffin (medullary) cells (M). X450.

67 Control section of the adrenal of the embryo shown in figures 60 - 66 incubated in media which lacked the substrate. Trace amounts of non-specific diformazan are present. X450.

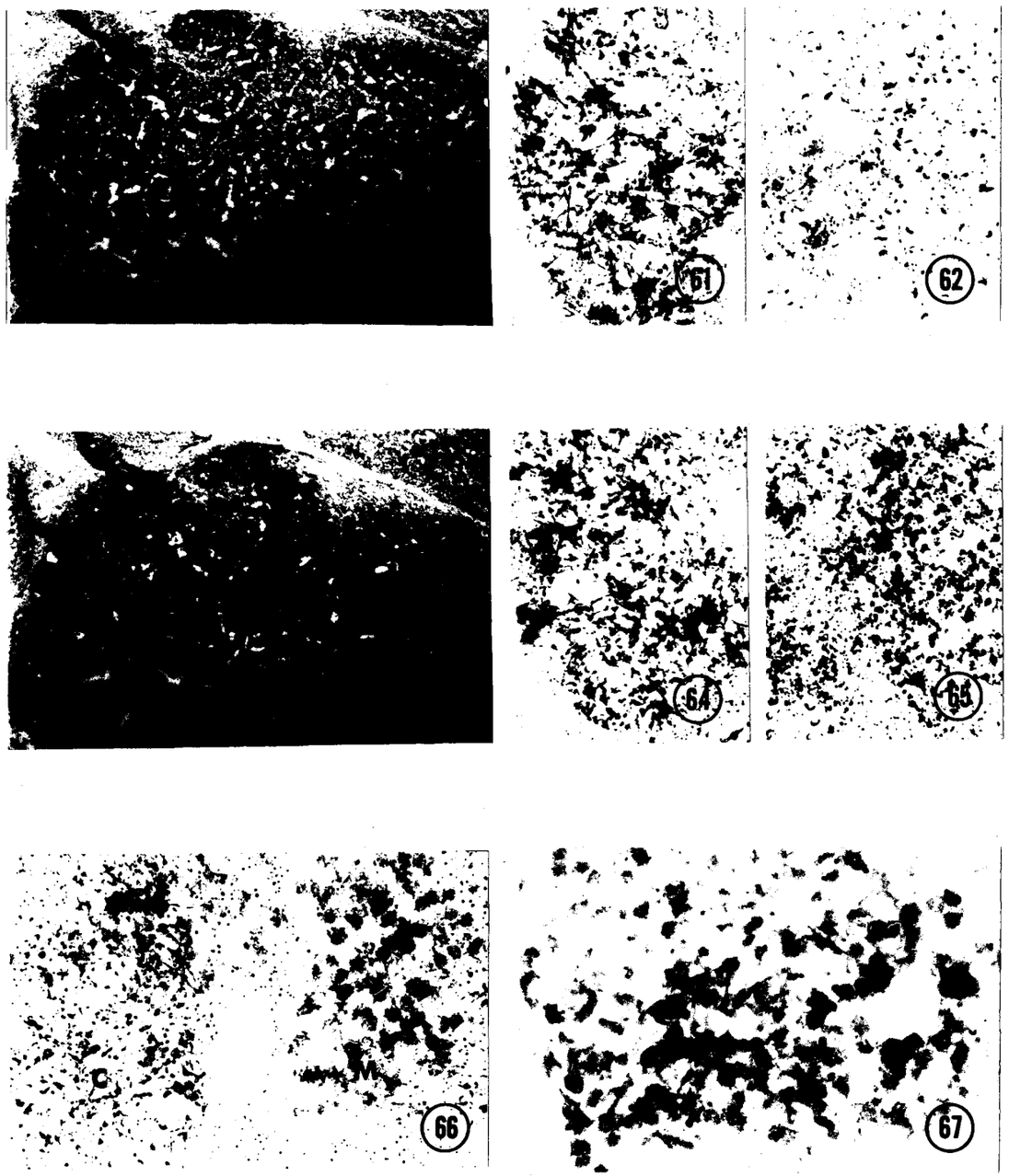


PLATE 24

EXPLANATION OF FIGURES

68 Diaphorase activity in the adrenocortical cells of a normal stage 25 (4.5 - 5 day) embryo. This activity is considerably higher than the P-3 -HSD activity (figure 67) and the DHA-3β-HSD activity (figure 70) of the same embryo. X450.

69 P-3β-HSD activity in the adrenocortical cells of the normal stage 25 (4.5 - 5 day) embryo shown in figure 68. X450.

70 DHA-3β-HSD activity in the adrenocortical cells of the normal stage 25 (4.5 - 5 day) embryo shown in figure 68. X450.

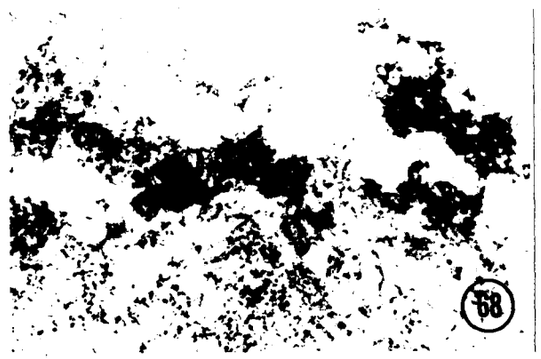
71 Diaphorase activity in a section of adrenal from a normal 10 day embryo. This activity is higher in the peripheral (P) than in the central zone (C). Diaphorase activity is considerably higher than the P-3β-HSD activity (figure 72) and the DHA-3β-HSD activity (figure 73) of the same embryo. X450.

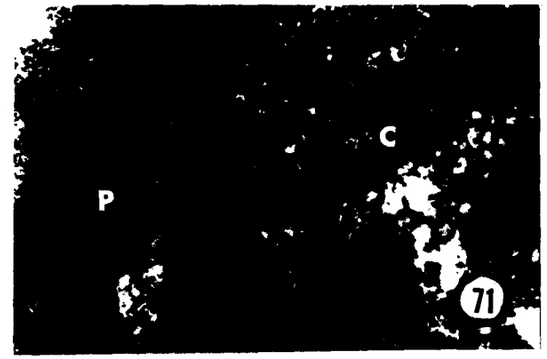
72 P-3β-HSD activity in a section of adrenal from the normal 10 day embryo shown in figure 71. This activity has the same distribution as the diaphorase activity, that is, high in the peripheral zone (P) and lower in the central zone (C).

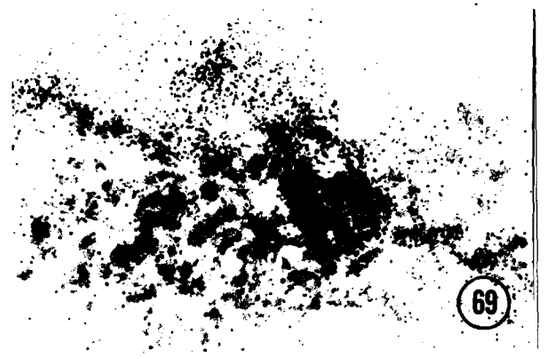
73 DHA-3β-HSD activity in a section of adrenal from the normal 10 day embryo shown in figure 71. This activity has the opposite distribution as that of the diaphorase activity, that is, higher in the central zone (C) than in the peripheral zone (P). The level of DHA-3β-HSD activity is less than that of diaphorase (figure 71).

74 Diaphorase activity in a cross section of a normal 20 day embryo. A narrow peripheral zone of high activity and a wide central zone of lower activity can be seen in the adrenal (A). There is also high activity in the medullary cords of the left ovary (O). X18.

75 Higher magnification of the diaphorase activity in the adrenal shown in figure 74. The peripheral zone (P) has higher activity than the central zone (C). X450.







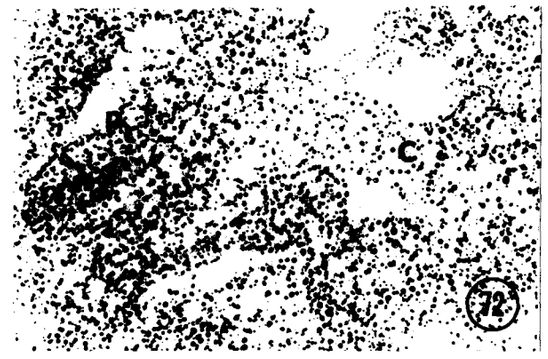


PLATE 25

EXPLANATION OF FIGURES

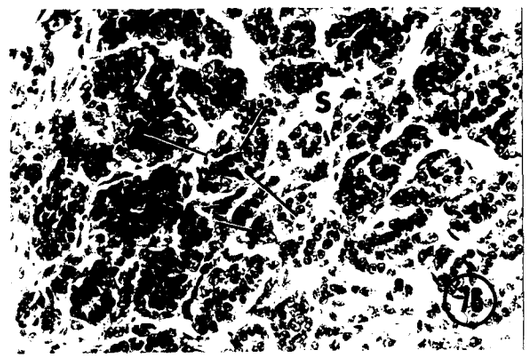
The adrenals shown in the photomicrographs in this plate were fixed in Bouin's fluid, paraffin embedded, sectioned, and stained with hematoxylin and eosin.

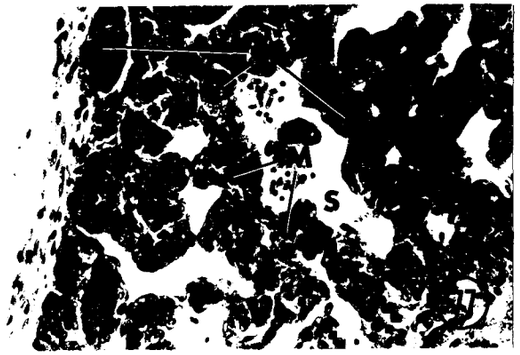
76 Section of the adrenal of a normal 10.5 day embryo. The cortical cells (C) are distributed throughout the gland in the form of irregularly arranged, frequently anastomosing, columns interspersed with groups of chromaffin (medullary) cells (M). Blood sinuses (S) separate the cortical cords. Compare figure 77. X250.

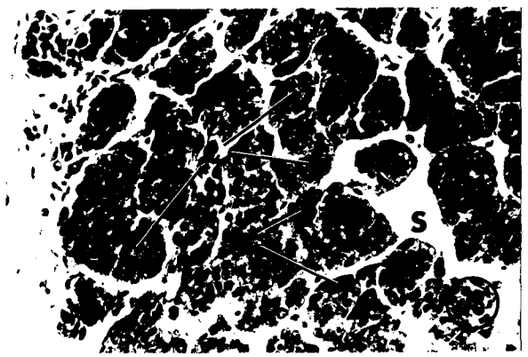
77 Section of the adrenal of a hypophysectomized 10.5 day embryo. The cortical cords (C) are slightly hypertrophied and reduced in number while the size of the groups of chromaffin (medullary) cells (M) are somewhat larger than normal. There is also an increase in the size of the blood sinuses (S). Compare figure 76. X250.

78 Section of the adrenal of a normal 17.5 day embryo. Normal appearing cortical cords (C), groups of chromaffin (Medullary) cells (M) and blood sinuses are shown. Compare figure 79. X250.

79 Section of the adrenal of a hypophysectomized 17.5 day embryo. The cortical cords (C) are hypertrophied and reduced in number and separated from one another by large accumulations of chromaffin (medullary) cells (M) and large blood sinuses (S). Compare figure 78. X250.







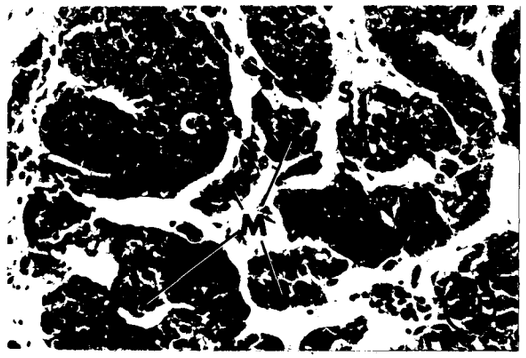


PLATE 26

EXPLANATION OF FIGURES

80 P-3β-HSD activity in the adrenal from a hypophysectomized 9 day embryo showing the absence of distinct cortical zones. Compare with section of adrenal of normal 9 day embryo shown in figure 43. X112.

81 DHA-3β-HSD activity in the adrenal of the hypophysectomized 9 day embryo shown in figure 80 showing the absence of distinct cortical zones. Compare with section of adrenal of normal 9 day embryo shown in figure 46. X112.

82 P-3β-HSD activity in the adrenal from a hypophysectomized 15 day embryo. The peripheral zone of high activity is wider and the central zone of lower activity is smaller than normal (see figure 54). The peripheral zone is incomplete due to large amounts of chromaffin tissue, separating the cortical cords. X56.

83 Higher magnification of the peripheral zone P-3β-HSD activity of 5 intensity shown in figure 82. Compare figures 84 and 88. X450.

84 Higher magnification of the central zone P-3β-HSD activity of 4 intensity shown in figure 82. Compare figures 83 and 88. X450.

85 DHA-3β-HSD activity in the adrenal of the hypophysectomized 15 day embryo shown in figure 82. The peripheral zone of low activity is wider and the central zone of high activity is smaller than normal (see figure 57). The peripheral zone is not complete due to large amounts of chromaffin tissue separating the cortical cords. X56.

86 Higher magnification of the peripheral zone DHA-3β-HSD activity of 4 intensity shown in figure 85. Compare figures 87 and 88. X450.

87 Higher magnification of the central zone DHA-3β-HSD activity of 5 intensity shown in figure 85. Compare figures 86 and 88. X450.

88 Control section of the adrenal of the embryo shown in figures 82 - 87 incubated in media which lacked the substrate. Trace amounts of non-specific diformazan are present. X450.

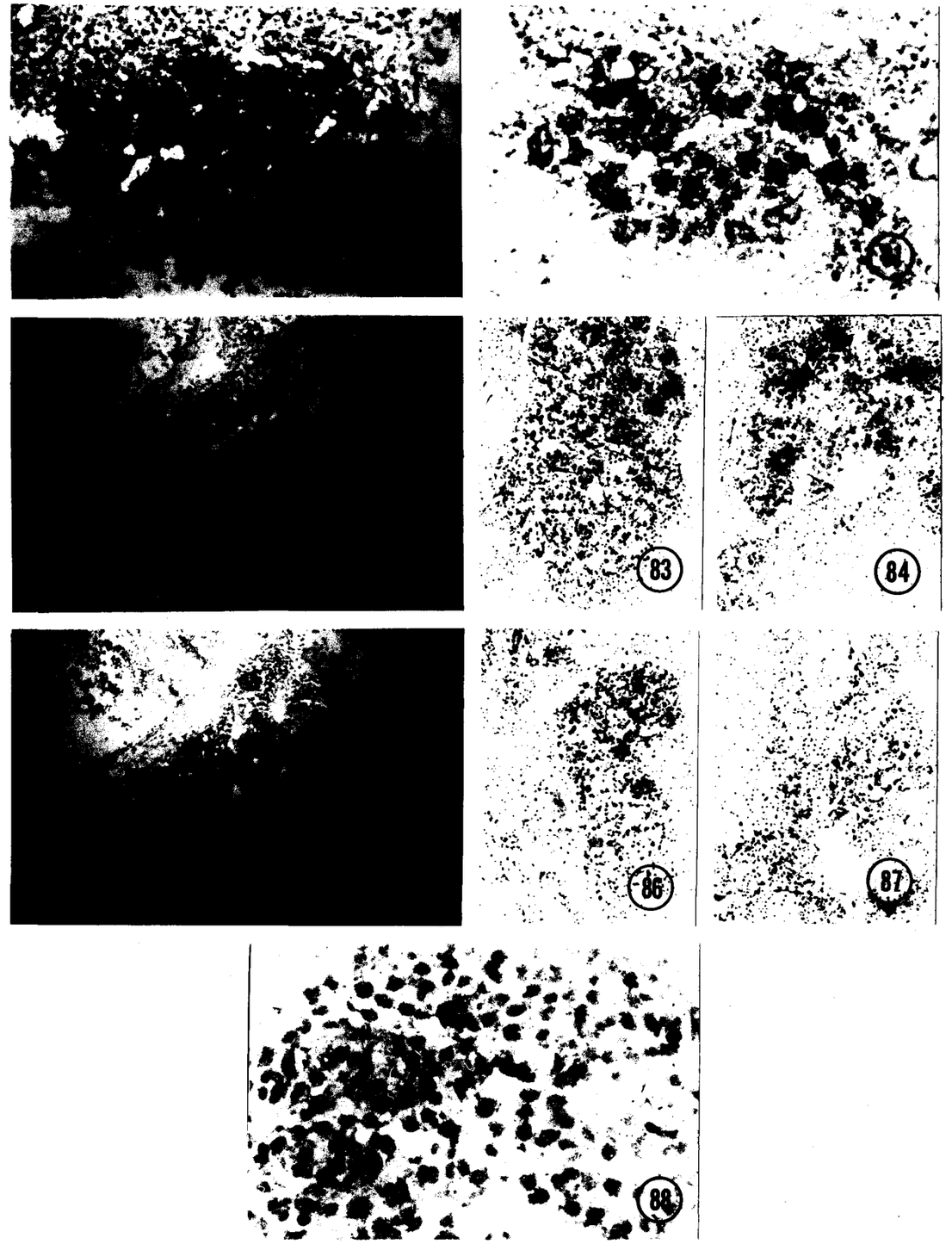


PLATE 27

EXPLANATION OF FIGURES

89 P-3β-HSD activity in the adrenal of a hypophysectomized 15 day embryo showing the absence of cortical zones (compare figures 90 and 91). Cortical cords are separated by large accumulations of chromaffin cells. Compare with section of adrenal of normal 15 day embryo shown in figure 54. X56.

90 Higher magnification of the P-3β-HSD activity in the periphery of the adrenal shown in figure 89. The peripheral P-3β-HSD is the same as the central P-3β-HSD activity shown in figure 91. X450.

91 Higher magnification of the P-3β-HSD activity in the central region of the adrenal shown in figure 89. Central activity is the same as that seen at the periphery (compare figure 90). The chromaffin tissue lacks P-3β-HSD activity. X450.

92 P-3β-HSD activity in the adrenal of a hypophysectomized 18 day embryo. Compare with normal adrenal shown in figure 60. Also see figures 93 and 94. X40.

93 Higher magnification of the peripheral zone P-3β-HSD activity of 6 intensity shown in figure 92. Compare figures 94 and 98. X450.

94 Higher magnification of the central zone P-3β-HSD activity of 4 intensity shown in figure 92. Compare with central zone activity of adrenal of normal embryo shown in figure 62. Compare figures 93 and 98. X450.

95 DHA-3β-HSD activity in the adrenal of the embryo shown in figure 92. Compare with normal adrenal shown in figure 63. Also see figures 96 and 97. X40.

96 Higher magnification of the peripheral zone DHA-3β-HSD activity of 5 intensity shown in figure 95. Compare figures 97 and 98. X450.

97 Higher magnification of the central zone DHA-3β-HSD activity of 4 intensity shown in figure 95. Compare figures 96 and 98. X450.

98 Control section of the adrenal of the embryo shown in figures 92 - 97 incubated in media which lacked the substrate. Trace amounts of non-specific diformazan are present. X450.

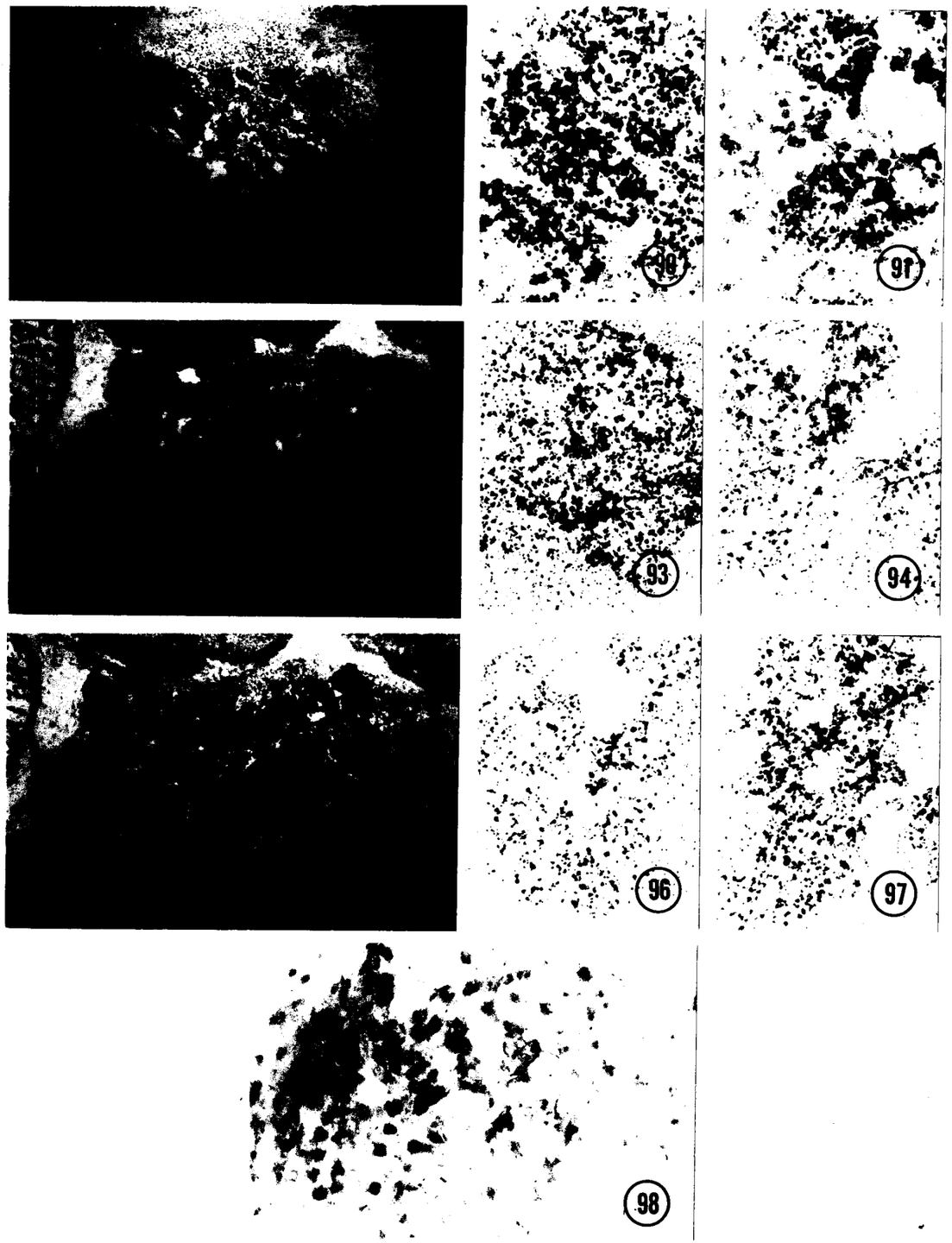


PLATE 28

EXPLANATION OF FIGURES

The tissues shown in the photomicrographs in this plate were fixed in Bouin's fluid, paraffin embedded, sectioned, and stained with hematoxylin and eosin.

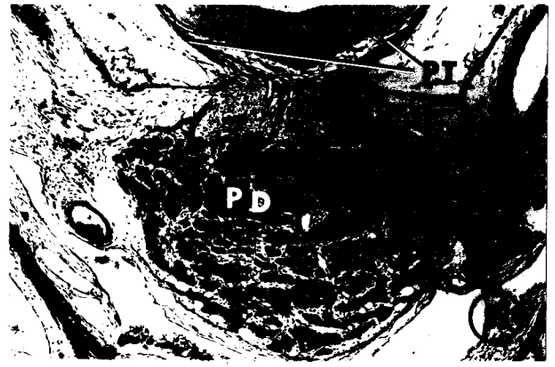
99 Cross section of the pituitary of a normal 17 day embryo. The pars distalis (PD) and pars tuberalis (PT) are shown. X56.

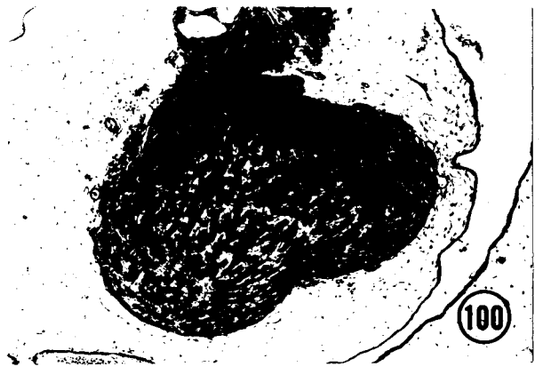
100 Adenohypophyseal graft recovered from the chorioallantoic membrane of a 14 day host hypophysectomized embryo. The graft contains well developed adenohypophyseal tissue. Compare figure 99. X56.

101 Adenohypophyseal graft recovered from the chorioallantoic membrane of a 17 day host hypophysectomized embryo. The graft contains well developed adenohypophyseal tissue. Compare figure 99.

102 Adenohypophyseal graft recovered from the chorioallantoic membrane of a 14 day host hypophysectomized embryo. The graft is undergoing degeneration. Compare with healthy graft shown in figure 100. X56.

103 Adenohypophyseal graft recovered from the chorioallantoic membrane of a 17 day host hypophysectomized embryo. The graft is undergoing degeneration. Compare with healthy graft shown in figure 101. X56.





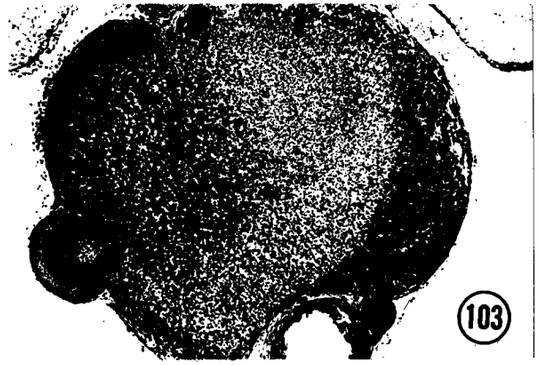


PLATE 29

EXPLANATION OF FIGURES

104 P-3β-HSD activity in the adrenal of a 10 day hypophysectomized embryo which received an adenohypophyseal chorioallantoic graft after 8.5 days of incubation. The adrenal resembles that of a normal embryo. Compare figure 43. X112.

105 Higher magnification of the peripheral zone P-3β-HSD activity of 6 intensity shown in figure 104. Compare figure 106. X450.

106 Higher magnification of the central zone P-3β-HSD activity of 4 intensity shown in figure 104. Compare figure 105. X450.

107 DHA-3β-HSD activity in the adrenal of the embryo shown in figure 104. The adrenal resembles that of a normal embryo. Compare figure 46. X112.

108 Higher magnification of the peripheral zone DHA-3β-HSD activity of 4 intensity shown in figure 107. Compare figure 109. X450.

109 Higher magnification of the central zone DHA-3β-HSD activity of 6 intensity shown in figure 107. Compare figure 108. X450.

110 P-3β-HSD activity in the adrenal of a 18 day hypophysectomized embryo which received an adenohypophyseal graft after 8.5 days of incubation. This adrenal resembles that of a normal embryo (see figure 60) rather than that of a hypophysectomized embryo (see figure 92). X40

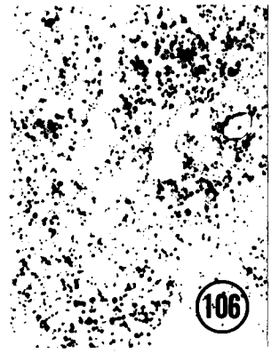
111 Higher magnification of the peripheral zone P-3β-HSD activity of 6 intensity shown in figure 110. Compare figure 112. X450.

112 Higher magnification of the central zone P-3β-HSD activity of 3 intensity shown in figure 110. Compare figures 62 and 94. X450.

113 DHA-3β-HSD activity in the adrenal of the 18 day embryo shown in figure 98. This adrenal resembles that of a normal embryo (see figure 63) rather than that of a hypophysectomized embryo (see figure 95). X40.

114 Higher magnification of the peripheral zone DHA-3β-HSD activity of 5 intensity shown in figure 113. Compare figure 115. X450.

115 Higher magnification of the central zone DHA-3β-HSD activity of 6 intensity shown in figure 113. Compare figure 114. X450.



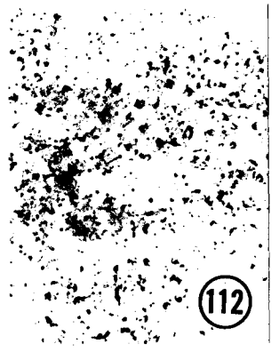



PLATE 30

EXPLANATION OF FIGURES

116 P-3β-HSD activity in the adrenal of a 10 day hypophysectomized embryo which received 1.0 IU of ACTH at 12 hour intervals. There is a peripheral zone of high activity and a cental zone of lower activity. The distribution of this activity resembles that of normal embryos (figures 43). X112.

117 Higher magnification of the peripheral zone P-3β-HSD activity of 6 intensity shown in figure 116. Compare figure 118. X450.

118 Higher magnification of the central zone P-3β-HSD activity of 5 intensity shown in figure 116. Compare figure 117. X450.

119 DHA-3β-HSD activity in the adrenal of the 10 day embryo shown in figure 116. There is a peripheral zone of low activity and a central zone of higher activity. The distribution of this activity resembles that of a normal embryo (see figure 46). X112.

120 Higher magnification of the peripheral zone DHA-3β-HSD activity of 4 intensity shown in figure 119. Compare figure 121. X450.

121 Higher magnification of the central zone DHA-3β-HSD activity of 5 intensity shown in figure 119. Compare figure 120. X450.

122 P-3β-HSD activity in the adrenal of a 18 day hypophysectomized embryo which received 1.0 IU of ACTH at 12 hour intervals. There is a narrow peripheral zone of high activity and a central zone of lower activity. The distribution of this activity resembles that of a normal embryo (see figure 60) rather than that of a hypophysectomized embryo (see figure 92). X40.

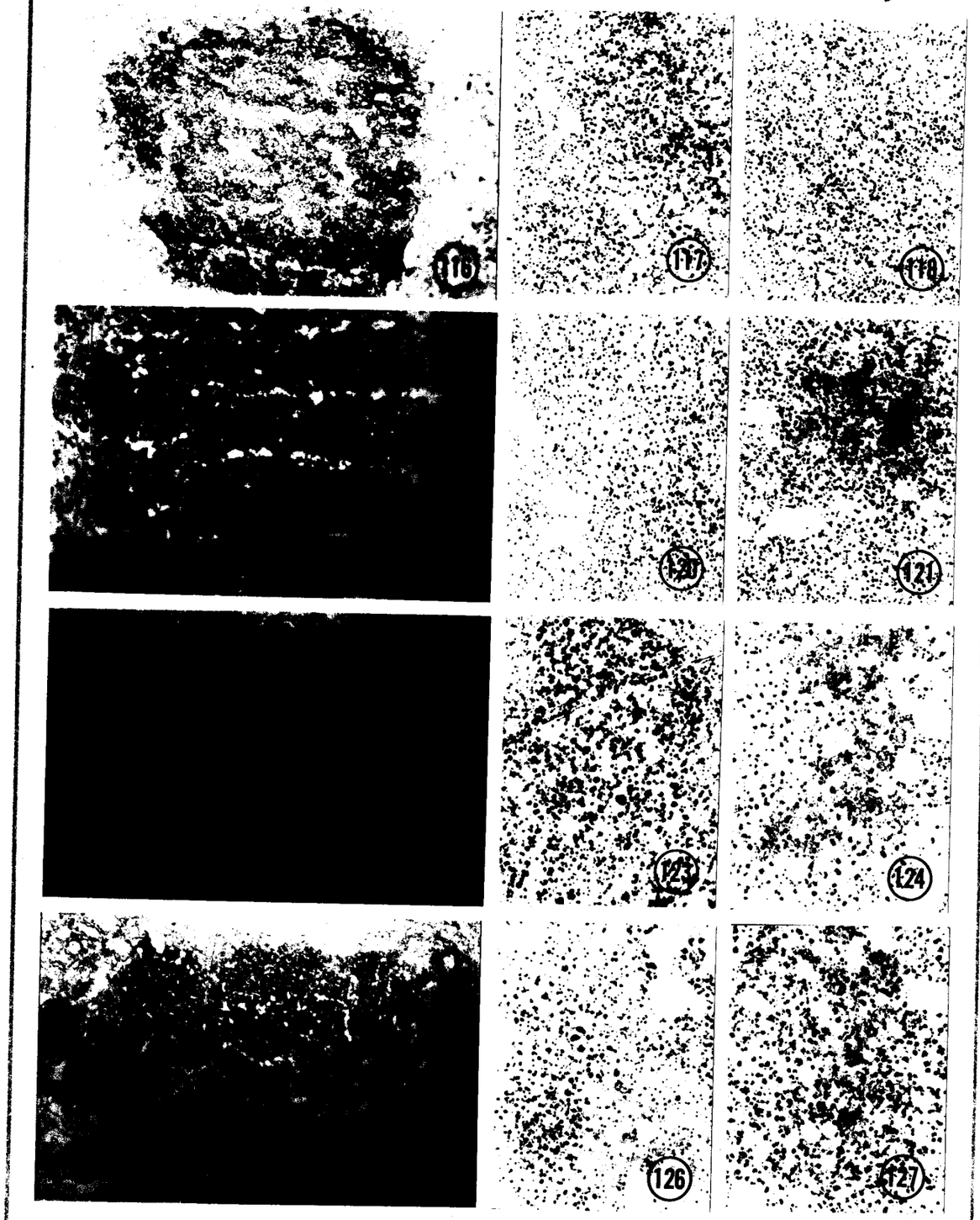
123 Higher magnification of the peripheral zone P-3β-HSD activity of 6 intensity shown in figure 122. Compare figure 124. X450.

124 Higher magnification of the central zone P-3β-HSD activity of 4 intensity shown in figure 122. Compare figure 123. X450.

125 DHA-3β-HSD activity in the adrenal of the embryo shown in figure 122. There is a narrow peripheral zone of low and a wide central zone of higher activity. The distribution of this activity resembles that of a normal embryo (see figure 63) rather than that of a hypophysectomized embryo.(figure 95). X40.

126 Higher magnification of the peripheral zone DHA-3β-HSD activity of 4 intensity shown in figure 125. Compare figure 127. X450.

127 Higher magnification of the central zone DHA-3β-HSD activity of 5 intensity shown in figure 125. Compare figure 126. X450.



APPROVAL SHEET

The dissertation submitted by Grover Charles Ericson has been read and approved by six members of the Faculty of the Graduate School.

The final copies have been examined by the director of the dissertation and the signatures which appear below verify the fact that any necessary changes have been incorporated, and that the dissertation is now given final approval with reference to content, form and mechanical accuracy.

The dissertation is therefore accepted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

May 22, 1972
Date

Signature of Adviser

Signature of Departmental chairman